In Vivo Evaluation of the D_1 Agonist PET Ligand R-[^{11}C]SKF 82957: Metabolism and Regional Brain Distribution in Animal Models and Humans

By

Robert A. Schwartz

A thesis submitted in conformity with the requirements for the degree of Master of Science in the Graduate Department of Pharmacology, University of Toronto

***In Vivo* Evaluation of the D_1 Agonist PET Ligand R-[^{11}C]SKF 82957: Metabolism and Regional Brain Distribution in Animal Models and Humans**

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Robert A. Schwartz
Graduate Department of Pharmacology, University of Toronto

ABSTRACT

Rationale: The dopamine D_1 receptor high-affinity (D_1^{HIGH}) state is believed to be the functional state of the receptor coupled to the G protein. Only agonist or partial agonist radioligands are capable of discerning the high- and low-affinity state with positron emission tomography (PET), while antagonists cannot. To date all PET neuroreceptor radioligands for imaging D_1 receptors have been antagonists, only capable of measuring the total receptor density. PET imaging of Parkinson's disease and schizophrenia with the D_1 antagonist [^{11}C]SCH 23390 has shown no changes in the striatum as compared to controls. With the use of a selective D_1 agonist radioligand, such as R-[^{11}C]SKF 82957, it may be possible to assess changes in the D_1^{HIGH} receptor *in vivo* in human subjects with PET. *Objectives*: The main objective of this research was to determine the suitability of R-[^{11}C]SKF 82957 for its use in human PET imaging. The metabolism of this radioligand was first ascertained in rat plasma and brain extracts, then in human plasma. with particular attention focused on the possible formation of radiolabeled metabolites with the potential to cross the blood brain barrier. PET imaging was performed in healthy human volunteers, and regional brain uptake and binding potential of striatal R-[^{11}C]SKF 82957 was examined. Two rat models of D_1 receptor supersensitivity (chronic D_1 agonist treatment with SKF 81297 and unilateral 6-hydroxydopamine (6-OHDA) lesions), were tested to establish the *in vivo* binding of R-

[^{11}C]SKF 82957 versus that of [^{11}C]SCH 23390. *Methods*: Reverse phase Sep Pak extraction of rat and human plasma followed by chromatographic analysis of the hydrophobic fraction was used in the metabolism studies. PET imaging in 11 subjects was analyzed for radioactivity distribution, age effects, and binding potential measurements. Rat regional brain uptake of R-[^{11}C]SKF 82957 and [^{11}C]SCH 23390 was evaluated following chronic SKF 81297 (0.5 mg/kg s.c., twice daily for 21 days, 7 days withdrawal), or unilateral 6-OHDA lesions of the right medial forebrain bundle. *Results*: Rat metabolic studies indicated no radiolabeled metabolites in brain extracts, and a low presence of polar metabolites in plasma (~86% unchanged R-[^{11}C]SKF 82957 at 30 min post-injection). Presence of metabolites in human plasma was also low (>85% unchanged R-[^{11}C]SKF 82957 at 80 min post-injection). Rapid striatal uptake (within 10 min) with a gradual washout was seen by PET in humans following R-[^{11}C]SKF 82957 administration. An approximate 0.9% per year decrease in the striatal binding potential was observed (age range 24-42 years). Good reproducibility and reliability in human R-[^{11}C]SKF 82957 PET imaging was achieved. As compared to controls, no significant difference in the R-[^{11}C]SKF 82957 and [^{11}C]SCH 23390 regional brain uptake was detected in the rats treated chronically with the full D_1 agonist SKF 81297 or following 6-OHDA lesioning. *Conclusions*: R-[^{11}C]SKF 82957 is the first dopamine PET agonist radioligand that shows significant uptake in the human basal-ganglia. Its metabolic profile, reproducibility and reliability in humans indicates that it may be used in PET. The lack of significant change in R-[^{11}C]SKF 82957 and [^{11}C]SCH 23390 brain uptake and region-to-cerebellum ratios in two experimental paradigms known to cause D_1 receptor supersensitivity, suggests that the cause of the enhanced response is due to changes downstream of the D_1 receptor.

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I dedicate this thesis to myself, because I deserve it, and to my parents... I told you I would finish.

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Robert A. Schwartz, Jean N. DaSilva, Paul J. Fletcher, Alan A. Wilson, and Sylvain Houle. **Effect of Chronic SKF 81297 Agonist and Unilateral 6-Hydroxydopamine Treatment on the In Vivo Binding of the D_1 Agonist R-[^{11}C]SKF 82957 in Rats.** *Journal of Neurochemistry* (to be submitted).

Jean N. DaSilva, Robert A. Schwartz, Douglas Hussey, Kevin Cheung, Alan A. Wilson, and Sylvain Houle. **In Vivo Human Brain Imaging with the Dopamine D_1 Agonist R-[^{11}C]SKF 82957 and Positron Emission Tomography.** *Neuro Report* (to be submitted)

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ABBREVIATIONS

5HT	5-hydroxytryptamine (serotonin)
6-OHDA	6-hydroxydopamine
AC	adenylyl cyclase
ALD-D	aldehyde dehydrogenase
ANOVA	analysis of variance
ATP	adenosine triphosphate
BBB	blood brain barrier
BP	binding potential
cAMP	3', 5'-cyclic adenosine monosphophate
COMT	catechol-O-methyltransferase
D_1^{HIGH}	D_1 high-affinity
D_1^{LOW}	D_1 low-affinity
DA	dopamine
DAG	diacylglycerol
DOPAC	3,4-dihydroxyphenylacetic acid
G protein	guanine nucleotide binding protein
GDP	guanosine diphosphate
G_i	inhibitory G protein
G_s	stimulatory G protein
GTP	guanosine triphosphate
GDP	guanosine diphosphate
HD	Huntington's disease

HPLC	high-performance liquid chromatography
HVA	homovanillic acid
IP3	inositol triphosphate
MAO	monoamine oxidase
MFB	medial forebrain bundle
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	magnetic resonance imaging
NA	noradrenaline
PD	Parkinson's disease
PET	positron emission tomography
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
ROI	region of interest
SZ	schizophrenia
SNpc	substantia nigra pars compacta
$t_{1/2}$	half-life
TD	tardive dyskinesia
TLC	thin-layer chromatography
TM	transmembrane

1.0 INTRODUCTION

INTRODUCTION

1.1 POSITRON EMISSION TOMOGRAPHY

Positron-emitting radionuclide-labeled tracers and positron emission tomography (PET) have been utilized for the study of many neuropsychiatric disorders. In particular, the dopamine (DA) system has been examined with a variety of PET radioligands for the assessment of postsynaptic receptors, presynaptic DA and vesicular transporters, and enzymes of DA metabolism. One of the benefits of PET imaging is that receptor properties can be measured *in vivo* in the living human brain as opposed to *in vitro* studies of postmortem tissues. Discrepant results with the use of postmortem tissues generally arise due to the application of differing assay techniques; this is because receptor function is sensitive to the incubation medium in which it is being expressed. For example, varying the concentrations of NaCl and GTP can greatly alter receptor efficacy. PET scans however obviate this need, as physiological conditions necessarily exist. Furthermore, individuals can be imaged before the onset of disease and throughout its progression. Likewise receptor properties can be assayed in drug naïve patients and subsequent to drug treatment. Therefore, changes in receptor properties can be compared within the same individual throughout the entire length of disease progression and treatment.

PET relies on the use of radioisotopes that undergo β^+ decay (e.g. ^{11}C, ^{13}N, ^{15}O, and ^{18}F). The common feature of these isotopes is that they all have too few neutrons as compared to their number of protons. Therefore, a proton is converted into a neutron, such that a positron (positively charged particle with the same mass as an electron) is released. The positron is emitted from the nucleus and travels at most a few millimeters before

colliding with an electron. The ensuing annihilation reaction produces two quanta of gamma rays (γ) each having 510 keV. The γ-photons fly off in opposite direction along the same path until they collide with the γ-detector. A series of detectors around the body establishes the location of the γ-source (Fig 1).

Analysis of radioactivity emitted from a single location during a human brain PET scan requires anatomical landmarks to verify brain structures. The method utilized to accomplish this task is termed coregistration. Briefly, a magnetic resonance image (MRI) of the brain that provides anatomical locations is overlaid that of the PET scan. Regions of interest (ROI) are then selected from the MRI, and computer software matches these to areas on the PET scan images. By this method the radioactivity located in discrete brain regions can be calculated using different mathematical algorithms. The index for receptor occupancy is termed the binding potential (BP) and represents the ratio of specific to nonspecific binding. Nonspecific binding being that observed in a region of the brain known to be deficient in the receptor of interest.

The radioligands utilized for PET have identical pharmacokinetic and pharmacodynamic properties as their non-radiolabeled analogues. Synthesis of these compounds is accomplished by the use of a cyclotron to produce the radioisotope followed by radio-chemistry techniques for incorporation into a precursor molecule. Depending on the radiotracer used, PET images related to biochemistry, metabolism, and function can be obtained.

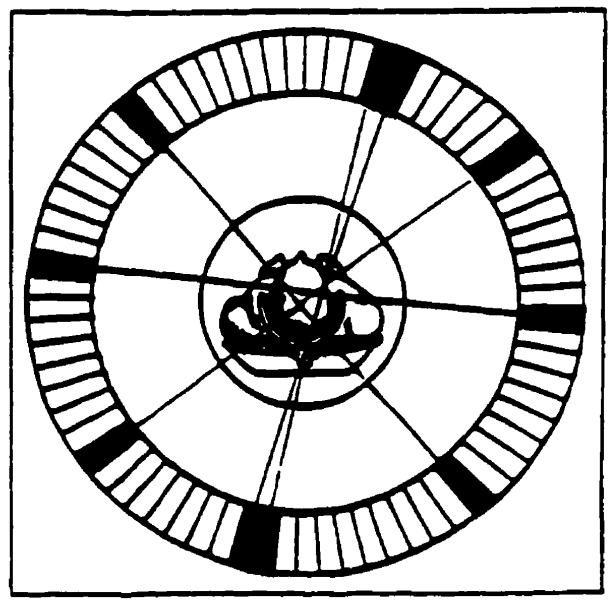

Fig 1. Schematic illustration of a positron camera

1.2 GENERAL BACKGROUND

The first evidence of monoamines in the central nervous system of various animal species was established in the 1950's (reviewed in Missale et al., 1998; Ungerstedt, 1971b), and demonstrated that noradrenaline (NA), DA and serotonin (5HT) existed in discrete brain regions. It took several more decades however to establish receptor classes for these endogenous neurotransmitters. In fact, only in 1979 did Kebabian and Calne (Kebabian and Calne, 1979) propose the existing DA receptor classification which continues to be used to this day. With the discovery of novel pharmacological agonists and antagonists for these various receptors, it was then possible to establish the physiological role played by each. Subsequently, the DA neuronal system has been implicated in many brain functions including cognition, motor function, and prolactin secretion, among others. In addition to this, the etiologies of several human neuropsychiatric brain disorders have been attributed, at least in part, to alterations in the DA neuronal system. Included in this is schizophrenia (SZ), tardive dyskinesia (TD), Huntington's (HD) and Parkinson's disease (PD), and drug addiction (reviewed in Clark and White, 1987; Davis et al., 1991; Hornykiewicz, 1966; Hyman, 1996; Joyce et al., 1988; Miller and Chouinard, 1993; Seeman et al., 1987; Woolverton and Johnson, 1992). New molecular biology and nuclear imaging techniques have extended our knowledge in these areas, yet, in all cited cases, the mechanism of dopaminergic involvement remains uncertain, and its elucidation occupies the work of many scientific laboratories.

1.3 DOPAMINE IN THE BRAIN

1.3.1 THE DOPAMINE NEURONAL SYSTEM

DA is the most abundant catecholamine in the mammalian brain, with two main neurological projections. The mesocorticolimbic system originates from the A10 DA cells of the ventral tegmental area (VTA) and projects to the nucleus accumbens (NAc), the olfactory tubercles and cortical areas. This pathway is implicated in reward and motivation. A second pathway, the nigrostriatal circuit, originates at the A9 DA cells of the substantia nigra and terminates at the striatum. This pathway is concerned with motor control.

1.3.2 THE DOPAMINE NEUROTRANSMITTER

DA is synthesized in dopaminergic neurons from the precursor amino acid tyrosine. Cytosolic tyrosine hydroxylase takes this precursor and forms L-DOPA which is then converted into DA by the aromatic amino acid decarboxylase enzyme. DA is concentrated in intracellular vesicles by the DA vesicular membrane transporter. The drugs reserpine and tetrabenazine inhibit the action of this transporter, which has the effect of reducing neuronal DA concentrations. Upon synaptic release of DA, termination of its action is accomplished by its presynaptic re-uptake via the plasma membrane DA transporter (inhibited by cocaine) or through its metabolism. In the brain, the two major products of DA metabolism are 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Elsworth and Roth, 1997).

1.3.3 THE DOPAMINE RECEPTORS

As stated, the current classification of the DA receptor is based on the observation that one population of DA receptors activated the effector enzyme adenylyl cyclase (AC) while another inhibited it (Kebabian and Calne, 1979). The D_1 subfamily was termed as that which activated AC while the D_2 subfamily was that which inhibited AC or is independent of this action (Seeman and Grigoriadis, 1987; Stoof and Kebabian, 1984). Novel pharmacological agents and molecular biological techniques have now established the existence of multiple receptor isoforms. The D_1-like family now includes the D_1 and D_5 receptors, while the D_2-like family encompasses the D_2, D_3, and D_4 receptors (Niznik and Van Tol, 1992). The D_1 receptor is most widely spread and expressed at the highest levels in the mammalian brain as compared to the other DA receptors. It can be found in the striatum, nucleus accumbens, olfactory tubercle, limbic system, hypothalamus, thalamus, and substantia nigra (Halldin et al., 1994; Meador-Woodruff et al., 1996; Mengod et al., 1992). Depending on the location within the brain, both pre- and post-synaptic D_1 receptors are found (for review see (Missale et al., 1998). Furthermore, recent evidence suggests that the majority of D_1-like and D_2-like receptors are expressed in separate neurons, although discrepancy in the literature exists (Joyce, 1991; Missale et al., 1998; Seeman et al., 1994). In the striatum, both D_1-like receptors are found on the medium spiny neurons, while the D_5 receptor is also located on the large aspiny neurons (Bergson et al., 1995a; 1995b). However, the D_5 receptor has greater concentration of sites in the frontal cortex, as compared to the D_1 receptor which has greatest density in the striatum (Sunahara et al., 1991). Subcellular separation of these receptors also exists, such that D_1 receptors are mainly detected on the dendritic spines while D_5 receptors are located on the dendritic shafts

(Bergson et al., 1995a; 1995b). So far only DA is able to differentiate between the D_1-like receptor members, with D_5 being approximately 10 times more sensitive to DA than the D_1 receptor (Sunahara et al., 1991).

1.3.4 STRUCTURE OF D_1-LIKE RECEPTORS

The D_1-like receptors are members of the metabotropic group of cell membrane receptors. They function through the activation of an intermediary guanine nucleotide binding protein (G protein). This G protein then interacts with an effector enzyme that elicits the intracellular response through a second messenger cascade.

The human D_1 receptor is located on chromosome 5 while the D_5 receptor can be found on chromosome 4. Unlike the D_2-like receptor family there are no introns within either D_1-like receptor sequences. Two D_5 pseudogenes also exist, which are 98% identical to each other and 95% identical to D_5. However, each codes for a truncated, nonfunctional form of D_5. The D_1-like receptors share 50% overall sequence homology, with 80% homology in the putative seven transmembrane domain (TM). Structurally, the D_1-like receptors have a short third intracellular loop and long carboxy terminal tail as compared to the D_2-like receptors (reviewed in Lachowicz and Sibley, 1997; Missale et al., 1998; O'Dowd, 1993; Seeman, 1995)). D_1-like receptors contain a cysteine residue located near the beginning of the carboxy terminus that probably serves to anchor the receptor to the membrane. Two more cysteine residues in extracellular loops 2 and 3 presumably function to stabilize the protein structure by forming disulfied bridges. Agonist binding occurs in the hydrophobic TM domain. An aspartate residue in TM3 is thought to interact with the amine

side chain of catecholamines, while two serine residues in TM5 have been implicated in hydrogen bonding to the hydroxyl groups of the catechol. Most likely other key TM residues are also involved in agonist binding. Like all 7TM receptors, G protein coupling takes place on the third intracellular loop with serine and threonine residues located here and on the carboxy tail probably involved in receptor regulation (reviewed in Lachowicz and Sibley, 1997; Missale et al., 1998; O'Dowd, 1993).

1.3.5 G PROTEIN AND SECOND MESSENGER PATHWAYS

Rodbell (1980) introduced the concept of the guanine nucleotide binding protein as a regulator of receptor action on the effector enzyme. Over the past 20 years, tremendous research has been conducted on the nature of this interaction and on the great variety of G proteins (reviewed in Birnbaumer, 1990; Gilman, 1987). The G protein heterotrimer is composed of an α-, ß- and γ- subunit (Dessauer, 1996).

Traditionally, the D_1 receptor was associated with the Gs protein and stimulation of AC (Clark and White, 1987). Coupling of D_1-like receptors to AC results in the production of 3',5'-cyclic adenosine monophosphate (cAMP), the intracellular second messenger. All putative D_1 agonists are classified according to their ability to stimulate cAMP. Once formed, cAMP exerts its effect by activating cAMP-dependent protein kinases (e.g. PKA). These enzymes are responsible for the phosphorylation of other target proteins within the cell, whose ultimate effect is to modify cellular metabolism and gene expression (Seeman and Grigoriadis, 1987).

In studies using striatal homogenates, the D_1 receptor has recently been linked to several other G protein isoforms. This includes Gi, Golf and possibly Gq (Kimura et al., 1995; Sidhu, 1990; Sidhu et al., 1991; Undie and Friedman, 1990; Wang, 1995; Yu et al., 1996). The intracellular effect of these potential associations and its physiological relevance remains controversial, as discrepancies in the literature exist. The use of various molecular genetic techniques in cell lines, reconstitution experiments, and tissue homogenates have contributed to this discordance. Interaction of D_1-like receptors to the functioning of K^+ channels, arachidonic acid, and Na^+-K^+-ATPase's in the brain is less well defined (reviewed in Missale et al., 1998). However, the interplay of D_1-like receptors with all these pathways has tremendous importance. D_1 agonists in several animal models have failed to show a correlation between agonist efficacy at AC activity (as measured *in vitro*) and the *in vivo* behavioral response (Arnt et al., 1992; Gnanalingham et al., 1995b; 1995c; Undie et al., 1994), suggestive of the presence of an alternative signal transduction pathway involvement.

The coupling of D_1 receptors and Gq was reported to result in the production of another group of intracellular second messengers termed inositol triphosphate (IP3) and diacylglycerol (DAG) from the breakdown of phosphatidylinositol-bisphosphate. This occurs through the action of the effector enzyme phospholipase C (PLC). The response of a cell to IP3 is to mobilize intracellular Ca^{2+} stores, and has many effects, including the activation of Ca^{2+}-binding proteins (e.g. calmodulin) and Ca^{2+}/Calmodulin-dependent protein kinases. On the other hand, DAG remains membrane bound and activates another set of enzymes termed protein kinase C (PKC). Phosphorylation of ion channels, receptors, and proteins would then ensue (Mitchell and Seeman, 1998). Although IP3 production has been clearly associated with D_1-like receptors in various tissue systems (Felder et al., 1989 ; Undie

and Friedman, 1990), in isolated cell lines D_1-like receptors have not been positively linked to this pathway (Kimura et al., 1995). Furthermore, the D_1 knockout mouse has been shown to exhibit functional association only to AC as opposed to PLC (Friedman et al., 1997). Therefore, controversy remains as to the exact nature of the D_1 receptor second messenger system.

1.3.6 RECEPTOR MODELS and D_1 HIGH-AFFINITY STATE

The mobile receptor theory proposed by Jacobs and Cuatrecasas (1976) states that at least two binding sites of different affinity exist for each hormone (neurotransmitter), although only a single receptor is involved. Furthermore, the biological response is correlated to the occupancy of the functional high-affinity receptor site by the neurotransmitter (De Lean et al., 1980; Jacobs and Cuatrecasas, 1976; Mackay, 1990). As stated previously the concept of G protein interaction with the receptor and effector was presented by Rodbell (1980). Introduced at the same time, and based on the study of the ß-adrenergic receptor, the ternary complex model seeks to explain this receptor-G protein-effector interaction, and the existence of two binding states within the same receptor (De Lean et al., 1980; Kent et al., 1980; Stadel et al., 1980).

The high-affinity state is described as that which occurs when the receptor is functionally coupled to the G protein, while the low-affinity state is that which occurs when the G protein is dissociated from the receptor. In the presence of GTP, this dissociation predominates and mediates the transition from the high- to the low-affinity state. Agonist occupancy of the receptor is thought to cause the substitution of GTP for GDP at the Gα

protein. The Gβγ complex then dissociates from the now active Gα-GTP that goes on to stimulate the effector enzyme. Inactivation occurs through the hydrolysis of GTP and reassociation of the G protein subunits with the receptor (Mackay, 1990). The high-affinity agonist state of the D_1 receptor is the functional state through which, following agonist binding, Gs protein activation occurs such that AC activity is stimulated.

The traditional model of receptor dynamics has recently been modified due to the observation that constitutively active and promiscuous receptors exist, that GDP can reduce agonist affinity, and that the Gβγ subunit has regulatory properties (Bond, 1997; Leff et al., 1997; Onaran et al., 1993). However, the definition of the high- and low-affinity states remains unchanged.

In vitro studies have demonstrated that the G protein-linked D_1 receptor exists in either a high- (D_1^{HIGH}) or a low-affinity (D_1^{LOW}) state (Hess et al., 1986b; Seeman and Grigoriadis, 1987). In fact, DA has a 10^4-fold increased affinity for D_1^{HIGH} as opposed to D_1^{LOW} (Seeman et al., 1989). Only agonists or partial agonists are capable of differentiating these receptor states, while antagonists cannot (De Lean et al., 1980; Kimura et al., 1995; Stadel et al., 1980). Therefore, D_1 antagonists bind with a single affinity to the total receptor population (Bmax), and are capable of only determining changes to the total receptor density (Roseboom et al., 1989; Kimura et al., 1995; Rubinstein et al., 1990). On the other hand, based on *in vitro* experiments, selective *in vivo* imaging of D_1^{HIGH} receptor can only be accomplished with the use of an agonist radioligand. Alterations in the D_1^{HIGH} receptor have been reported in human neuropsychiatric disorders and in animal models of human brain disorders. For example, Mamelak et al (1993) showed a significant increase in the proportion of D_1^{LOW} receptors and a significant enhancement in the affinity of D_1^{HIGH} in

postmortem studies on human SZ sufferers. Likewise, Rubinstein et al. (1990) presented a 51% increase in the proportion of D_1^{HIGH} following chronic reserpine treatment in mice. Therefore, an agonist should be more appropriate to image the functional D_1^{HIGH} receptor with PET in both normal human subjects and in diseased brains. The agonist SKF 82957 was selected as a potential marker of the D_1^{HIGH} receptor due to its appropriate *in vitro* pharmacological binding profile. We hypothesize that this *in vitro* binding characteristic occurs *in vivo* as well.

1.4 NEUROPSYCHIATRIC DISORDERS AND D_1 RECEPTORS

1.4.1 PARKINSON'S DISEASE

Traditionally, the loss of DA neurons from the substantia nigra pars compacta (SNpc) to the striatum (caudate-putamen) has defined this disease (Strange, 1993). More recently however, PET has revealed that the earliest change is characterized by a loss of DA nerve terminals in the posterior putamen (Guttman et al., 1997). This is followed by a progression of denervation toward the caudate head, eventually encompassing the entire striatum. Examination of DA levels in the striatum using the PET radiotracer [^{18}F]-6-F-Dopa indicates an eventual depletion of this neurotransmitter (Donnan et al., 1991).

Clinical symptoms manifest as rigidity, resting tremor, and inability to initiate movement. The progression of this disease in humans leads to further motor dyskinesia, cognitive decline and may eventually result in dementia (Hurtig, 1997). Approximately 5% of the population over the age of 65 are expected to acquire this neurodegenerative disorder (Goldberg et al., 1998). The most common therapy is receptor stimulation following L-

DOPA treatment, which is usually administered p.o. and converted in the brain to DA by the enzyme aromatic amino acid decarboxylase in the remaining DA neurons of the nigrostriatal tract, and released at the synapse. Often the addition of monoamine oxidase (MAO-B) inhibitors (e.g. Selegiline) and peripheral decarboxylase inhibitors (e.g. Carbidopa) supplement this drug. Unfortunately, pharmacological treatment of the disease has limited value over time. As disease progression continues, even with drug therapy, an eventual "on-off" phenomenon is displayed where motor fluctuation is no longer correlated with drug plasma levels, and can no longer be effectively treated. In order to lengthen the period of time before this occurs, much research has focused on the use of selective and/or mixed D_1/D_2 agonists before L-DOPA treatment is initiated (Jenner, 1995; Stern, 1997; Watts, 1997).

Discrepancy in the literature exists as to the status of D_1 receptors in this disease. *In vitro* studies utilizing [^{3}H]SCH 23390 have shown increases and/or no changes in striatal D_1 receptor Bmax (Cortés et al., 1989b; Seeman and Grigoriadis, 1987). Most inconsistencies are a result of the drug treatment status of patients before receptor measurement (Mash et al., 1998). *In vivo* studies with the PET radiotracer [^{11}C]SCH 23390 have shown no changes in striatal D_1 receptor Bmax (Rinne et al. 1990b ; Shinotoh et al., 1993).

1.4.2 SCHIZOPHRENIA and TARDIVE DYSKINESIA

Neuroleptic use in the treatment of SZ has mainly focused on the blockade of D_2 receptors (Seeman and Van Tol, 1995). This has proven effective in treating the hallucinations, delusions and psychomotor unrest associated with the disease (Deniker,

1990). However the possibility of encountering unwanted side effects, exists with increased neuroleptic use. Included in these negative symptom side effects is the onset of extrapyramidal motor fluctuations characteristic of PD's and tardive dyskinesia (TD) (Hietala et al., 1990). To combat this obstacle, a new class of "atypical" antipsychotics has been developed (e.g. clozapine). D_1 receptor occupancy by this new class of drugs is much higher as compared to traditional neuroleptics (Farde et al., 1992; Farde et al., 1989).

Current PET research using [^{11}C]SCH 23390 indicates no change in striatal D_1 receptor Bmax in SZ (Sedvall, 1992). However, a decrease in D_1 receptor density was seen in the prefrontal cortex of drug naïve SZ as compared to controls (Okubo et al., 1997). Due to the dopaminergic hyperactivity associated with this disease, an expected decrease in the proportion of D_1 high-affinity (D_1^{HIGH}) sites would be hypothesized to occur. In fact, this decrease in the proportion of D_1^{HIGH} receptors was observed in the postmortem study of schizophrenia patients as compared to controls (Mamelak et al., 1993).

The involuntary oro-facial dyskinesia associated with TD is often seen as a side effect of conventional neuroleptic therapy in SZ. These dyskinetic movements may also include the limbs and trunk. In both rats and monkeys this event occurs following prolonged direct D_1 agonist stimulation (Lublin, 1995; Lublin et al., 1992; Miller and Chouinard, 1993). The reduced occurrence of this extrapyramidal motor fluctuation with the use of atypical antipsychotics is partly attributed to their greater D_1 blockade and that of 5-HT_{2A} (Casey, 1989).

1.4.3 HUNTINGTON'S DISEASE

Huntington's disease (HD) is a genetically determined neurological disorder resulting in choreiform movements. The pathology associated with this disease is severe neuronal degradation in the basal ganglia and the cerebral cortex (Spokes, 1981). D_1 receptor density has been shown to be decreased (Cross and Rossor, 1983; Filloux et al., 1990).

1.4.4 SUBSTANCE ABUSE

Increased dopaminergic tone is evident with the administration of several non- related compounds commonly abused by humans (e.g. cocaine, ethanol, nicotine) (Di Chiara and Imperato, 1988). For example, the actions of both cocaine and amphetamine are related to their interaction with the plasma membrane DA transporter, such that an increase in synaptic DA is achieved (Hyman, 1996). D_1 receptors are known to be involved in the reinforcing and behavioral effects of these drugs, as in various animal models full D_1 agonists will substitute for these substances of abuse (Grech et al. 1996; Henry and White 1991; Spealman et al. 1991; Weed et al. 1997; Weed and Woolverton 1995; May, 1992).

1.5 RECEPTOR REGULATION

1.5.1 DESENSITIZATION

Agonist activation of metabotropic receptors not only initiates an effector response, but also launches a regulatory process that results in the loss of cellular response to prolonged agonist stimulation. This occurs either through the downregulation of total receptor numbers (at the level of gene expression), or through the process of desensitization.

Homologous desensitization involves a loss of agonist responsiveness limited to the receptor being stimulated. Generally it occurs through the rapid phosphorylation of the receptor at serine and threonine residues located on the third intracellular loop and the carboxy terminus. This phosphorylation is commonly produced by specific G protein receptor kinases. Following phosphorylation of the receptor, arresting proteins attach and induce receptor-G protein uncoupling. A similar mechanism is utilized for heterologous desensitization. However, in this case, phosphorylation is conducted by protein kinases (e.g. PKC and PKA) not specific to a particular G protein; typically activated by the intracellular second messenger system. This allows for the desensitization of receptors that are not directly stimulated by agonist. Following these processes, receptor sequestration and internalization occurs, which presumably functions to resensitize the receptors (Ferguson et al., 1998).

1.5.2 SUPERSENSITIZATION

The modifications that lead to a supersensitized receptor response are less clear. Although an upregulation of total receptor numbers is a plausible explanation, it does not always occur. Other possible explanations include an enhanced G protein-receptor coupling such that an increase in the proportion of receptors in the high-affinity state is achieved. This would account for an augmented agonist response while not necessitating an increase in the total density of the receptor. Quantification of such a change would require the use of an agonist, since antagonists cannot differentiate between the high- and the low-affinity states. In theory therefore, a D_1 agonist such as R-[C^{11}]SKF 82957 could be used *in vivo* to

determine differential regulation of D_1 receptors in experimental paradigms known to cause supersensitization, as compared to the D_1 antagonist [C^{11}]SCH 23390. Other mechanisms that have been proposed seek to explain supersensitization by looking at components of the signal transduction pathway downstream of the receptor, such as increased G protein coupling efficiency and enhanced AC activity. Amplification at one or more of these steps can explain the increased responsiveness. As described below a number experimental models have been shown to cause D_1 receptor supersensitization.

1.5.2.1 CHRONIC DIRECT/INDIRECT AGONIST TREATMENT

Animal studies based on the administration of either direct acting D_1 receptor agonists (e.g. SKF 38393, SKF 81297) or indirect agonists (e.g. cocaine, amphetamine) have provided similar results. Generally, acute stimulation of DA receptors, such as an amphetamine challenge, results in homologous desensitization (Roseboom and Gnegy, 1989). However, chronic administration of these compounds followed by a specified period of withdrawal has consistently shown a supersentized D_1 response. For example, challenge with the partial D_1 agonist SKF 38393 induced an increased frequency of tongue protrusions seven days following a 14-day chronic cocaine treatment in rats. This reaction was not observed after only 4 hours withdrawal (Neisewander et al., 1996). Furthermore, iontophoretic application of the full D_1 agonist SKF 81297 resulted in an enhanced inhibitory effect on striatal neurons (Hu et al., 1992), as measured by single unit recordings. This was only observed one-week following chronic SKF 81297 (twice daily for 21-days) drug treatment in rats. These results,

indicate that the pharmacological regimen and length of withdrawal period are critical factors for the observation of supersensitivity.

1.5.2.2 6-HYDROXYDOPAMINE LESIONING

6-Hydroxydopamine (6-OHDA) is a catecholamine specific neurotoxin that selectively depletes NA and DA, while causing little damage to other neuronal systems (Breese and Traylor. 1970). Upon intracranial injection it enters the target cell through the catecholamine plasma membrane reuptake transporter. The mechanism of neurotoxicity is by free radical formation and inhibition of the mitochondrial respiratory chain complexes I and IV (Glinka et al., 1997). Injection of this compound into the medial forebrain bundle (MFB) causes catecholamine denervation in the striatum causing hemiparkinsonism (Przedborski et al., 1995), and is often used as an animal model for this disease.

An important breakthrough in the use of 6-OHDA came from Ungerstedt who demonstrated that the extent of rotation induced by amphetamine or apomorphine challenge correlated with the extent of degeneration in the DA system (Ungerstedt, 1971c). Accordingly, a unilaterally lesioned rat challenged with amphetamine will rotate in an ipsilateral (towards the lesioned side) direction, while an apomorphine challenge will result in contralateral (towards the nonlesioned side) rotation. Amphetamine induced rotation occurs because of the increase in dopaminergic tone at the nonlesioned striatum by the remaining nigrostriatal neurons, such that stimulation of the innervated receptor dominates. The induction of contralateral rotation by the mixed D_1/D_2 agonist apomorphine indicates that the receptors on the ipsilateral hemisphere are supersensitive (Hudson et al., 1993;

Ungerstedt, 1971a). Note that D_1 specific agonists can also initiate contralateral turning (Arnt and Hyttel, 1984; Gnanalingham et al., 1995b; 1995c; Matsuda et al., 1992).

The total density of D_1 receptors following unilateral lesioning has been analyzed by several different *in vitro* techniques producing discrepant results. This includes striatal D_1 receptor upregulation (Iwata et al., 1996; Porceddu et al., 1987), downregulation (Joyce, 1991), and no change (Graham et al., 1990; Lawler et al., 1995; Trugman et al., 1990). Regardless of these findings, the behavioral supersensitization as seen with agonist stimulation is observed in all cases.

1.5.2.3 CHRONIC RESERPINE

Reserpine blocks the function of the catecholamine vesicular membrane DA transporter such that synaptic DA levels are reduced. This results in sedation, hypokinesis, rigidity and tremor, which resembles PD. Increased AC activity is induced following this form of DA depletion (Arnt, 1985), however, no change in the D_1 total density is observed (Missale et al., 1989). Nonetheless, Rubinstein et al. (1990) did show an increase in the proportion of D_1 receptors in the high-affinity state, thus denoting a possible mechanism for the enhanced D_1 receptor supersensitivity, accounting for the increased AC activity.

1.5.2.4 MPTP LESIONS

Structurally resembling pethidine (a.k.a. meperidine), 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes symptoms virtually identical to PD (Kaakkola and Teravainen, 1990). Unlike 6-OHDA, this drug can be administered peripherally, however

it results in many serious systemic side effects, and is generally avoided. Typically, intracranial injections or infusions through the internal carotid artery are performed. Enhanced responsiveness to some D_1 agonists is observed following MPTP lesioning (Gnanalingham et al., 1995a; Vermeulen et al., 1993), and both up- and down-regulation of D_1 receptors are reported (Gnanalingham et al., 1993; Pifl et al., 1992a). Note that a species difference exists, such that rats are relatively resistant to MPTP (Mokry, 1995).

1.5.2.5 CHRONIC D_1 ANTAGONISTS

The selective D_1 antagonist SCH 23390 has been utilized in several drug paradigms to induce receptor supersensitization. Behavioral observations following chronic antagonist treatment indicates an enhanced stereotypy to the partial D_1 agonist SKF 38393 and increased locomotion (Hess et al., 1986a). AC activity was also increased following specific agonist challenge (Hess et al., 1986a). Although discrepancies exist, an upregulation of D_1 receptors is generally reported (Braun et al., 1997; Creese and Chen, 1985; Lappalainen et al., 1992). Interestingly, Hess et al (1986a) reports that *in vitro* measurements of the proportion of D_1 high-affinity sites in the striatum does not change following chronic SCH 23390 treatment.

1.6 RESEARCH OBJECTIVES

1.6.1 MAIN OBJECTIVE

The **main objective** of this research project is to (1) determine the metabolic profile of the novel PET D_1 agonist radioligand R-[^{11}C]SKF 82957 in rats and humans, (2) determine the suitability of this radiotracer for human PET studies, and (3) establish the

ability of R-[^{11}C]SKF 82957 to detect *in vivo* changes in D_1 receptor properties induced by dopaminergic manipulation in rats. The **ultimate objective** of the research project is to use R-[^{11}C]SKF 82957 to qualify *in vivo* the density of the functional D_1^{HIGH} receptor in normal and diseased human brains using PET.

1.6.2 HYPOTHESIS

1.6.2.1 GENERAL HYPOTHESIS

The general hypotheses of this research project include:

1. The D_1 agonist R-[^{11}C]SKF 82957 selectively binds *in vivo* to the high-affinity state of D_1 receptors.
2. The D_1 antagonist [^{11}C]SCH 23390 selectively binds *in vivo* to the total density of D_1 receptors.
3. R-[^{11}C]SKF 82957 and [^{11}C]SCH 23390 binding are differentially regulated in response to dopaminergic manipulations.

1.6.2.2 WORKING HYPOTHESIS

The working hypotheses of this project are:

1. R-[^{11}C]SKF 82957 shows no radiolabeled metabolite in rat brain.
2. R-[^{11}C]SKF 82957 binds to the striatum in humans and decreases with age.
3. R-[^{11}C]SKF 82957 provides reproducible and reliable PET data.
4. Following chronic treatment with the D_1 agonist SKF 81297 and unilateral 6-OHDA lesioning in rats, an increase in the binding of R-[^{11}C]SKF 82957 is observed in the rat striatum, and that of [^{11}C]SCH 23390 is unchanged.

1.6.3 SPECIFIC AIMS

The specific aims of this research project are:

1. To determine the metabolism of R-[^{11}C]SKF 82957 in rat and human plasma, with the focus on radiolabeled products.
2. To check for the presence of radiolabeled metabolites in rat brain extracts.
3. To image the brain of healthy human volunteers with R-[^{11}C]SKF 82957 and PET.
4. To assess the reproducibility and reliability of the PET data.
5. To study the *in vivo* binding of R-[^{11}C]SKF 82957 in rats following chronic treatment with SKF 81297 and unilateral 6-OHDA lesioning.

The purpose of this research is to assess the suitability of the novel D_1 agonist R-[^{11}C]SKF 82957 for use in human PET imaging studies, and test its ability to bind differently as compared to [^{11}C]SCH 23390 in the striatum of rats exhibiting D_1 receptor supersensitivity. Currently, no other selective D_1 receptor agonist PET radioligand exists. All PET studies performed in the past have investigated the D_1 receptor utilizing radiolabeled antagonists. Unfortunately, antagonists cannot differentiate between the high- and the low-affinity states of the receptor, and only an agonist or partial agonist can.

Human and animal models of neuropsychiatric disorders have previously shown changes in the proportion of D_1 receptors in the high-affinity state, while indicating no change in the total density of the receptor. If successful *in vivo* PET studies of the D_1

receptor in humans with R-[^{11}C]SKF 82957 will enable the imaging of the D_1^{HIGH} receptor state in various neuropsychiatric diseases including PD, SZ, TD, and drug addiction.

1.7 CHAPTER DESCRIPTIONS

1.7.1 CHAPTER 2

The R-[^{11}C]SKF 82957 enantiomer was synthesized, and its *in vivo* brain uptake and binding characteristics were compared to that of the racemic R/S-[^{11}C]SKF 82957 radioligand. It is expected that the R-enantiomer will display a greater signal-to-noise ratio as compared to the racemic form. Competition studies with D_1, D_2, and 5-HT_2 receptor antagonists are also performed to assess the binding selectivity of the pure enantiomer. Rat plasma extractions were performed with C_{18} Sep Paks and the eluted hydrophobic fractions were assayed by thin layer chromatography (TLC) for the presence of radiolabeled metabolites. Rat brain extracts were also analysed by TLC. Primary interest was placed on the detection of any radiolabeled metabolite that has the potential to cross the blood brain barrier (BBB).

1.7.2 CHAPTER 3

Results of the first series of R-[^{11}C]SKF 82957 human PET scan images are described. Striatal uptake and BP results are analyzed. BP values were calculated by two separate reference tissue techniques and compared to ascertain which has greatest suitability. Correlation between striatal BP and age is determined. Scan reproducibility and reliability is

assessed. Human plasma was assayed for the presence of radiolabeled metabolites having the potential to cross the BBB, using the same techniques as described in chapter two.

1.7.3 CHAPTER 4

Two pharmacological techniques are utilized to induce D_1 receptor supersensitivity in rats. Chronic D_1 agonist treatment and 6-OHDA lesioning were performed. One of our hypotheses states that an increase in the proportion of D_1^{HIGH} receptors, with or without a change in total density, may account for the receptor supersensitivity. In light of this, rats were assayed with R-[^{11}C]SKF 82957 and [^{11}C]SCH 23390 for changes in brain uptake and region to cerebellum ratios following these two treatment paradigms.

2.0 Dopamine D_1 Agonist *R*-[^{11}C]SKF 82957: Synthesis and *In Vivo* Characterization in Rats

Jean N. DaSilva, Robert A. Schwartz, Eric R. Greenwald,

Celia M. Lourenco, Alan A. Wilson and Sylvain Houle.

Submitted to: Nuclear Medicine and Biology

The experiment described in this paper required the assistance of at least five people in order to be carried out successfully. I assisted in the performance of all studies described herein and in the preparation of this manuscript. However, only the rat plasma and brain metabolite analysis, including experimental thought, data analysis, statistics and figures are presented as part of this thesis. **The chemistry, time course, *in vivo* competition, and dosimetry studies will therefore not be discussed outside of this chapter.**

2.1 ABSTRACT

The active enantiomer *R*-SKF 82957 was labeled with ^{11}C by *N*-[^{11}C]methylation of the full D_1 agonist *R*-SKF 81297, using [^{11}C]methyl iodide in the presence of N-ethyldiisopropylamine, in high specific activity, radiochemical purity and yields. Compared to the D_1 agonist *R/S*-[^{11}C]SKF 82957, *R*-[^{11}C]SKF 82957 showed higher binding in the D_1 rich regions, such as striatum and olfactory tubercles (~1.7 times), thereby improving the tissue contrast. *R*-[^{11}C]SKF 82957 exhibited high *in vivo* binding selectivity for D_1 receptors in rats, since only high doses of D_1 competitors, but not D_2 or 5-HT_2 blockers, significantly reduced the radioactivity levels in all brain areas. No labeled metabolites were detected in rat brain. These results indicate that *R*-[^{11}C]SKF 82957 will provide more sensitive measurements of D_1 receptors in *in vivo* studies than the racemic mixture.

2.2 INTRODUCTION

Alteration of dopamine (DA) activity has been implicated in the pathophysiology of several neuropsychiatric disorders, and in drug dependence. Traditionally, DA receptors were divided into two major groups: D_1 and D_2 receptors (Kebabian and Calne, 1979). Both receptors are primary targets for drugs used to treat many psychomotor disorders, and are involved in the actions of drugs of abuse (Clark and White, 1987; Waddington and O'Boyle, 1989; Woolverton and Johnson, 1992). D_1 receptors exist in two interconvertible states exhibiting either high- or low-affinity for agonists or partial agonists, while antagonists do not differentiate between these states and bind to the total number of D_1 receptors (Leff et al., 1985; Rubinstein et al., 1990; Seeman and Grigoriadis, 1987). Therefore, only D_1 agonist and partial agonist radiotracers have the potential to selectively assess the *in vivo* density of the functional high-affinity sites of D_1 receptors using positron emission tomography (PET). Generally, the binding of DA (or a D_1 agonist) to the high-affinity state of D_1 receptors activates the receptors, which through functional coupling to a stimulatory guanine nucleotide binding protein (G-protein, Gs), stimulates adenylyl cyclase (AC) activity leading to the formation of cAMP and subsequently to characteristic D_1 dopaminergic effects (Kimura et al., 1995; Seeman and Grigoriadis, 1987; Sidhu, 1988). D_1 agonists have also been shown to stimulate phospholipase-C, phosphoinositide activities, and possibly other signaling pathways (Kimura et al., 1995; Undie et al., 1994). In fact, a poor correlation was reported between agonist efficacy to stimulate AC and certain behavioral effects produced by a series of D_1 agonists in unilateral 6-hydroxydopamine lesioned rats, which may indicate a significant role for signaling pathways other than AC (Gnanalingham et al., 1995c).

Inconsistent results have been reported in postmortem *in vitro* studies with respect to the changes in striatal DA D_1 receptor densities in schizophrenia (SZ), Parkinson's disease (PD), and in the striatum of cocaine treated animals (Alburges et al., 1993; Guttman, 1992; Hess et al., 1987; Mayfield et al., 1992; Seeman et al., 1987). Clinical use of the D_1 antagonist [^{11}C]SCH 23390 and PET showed no difference in striatal D_1 receptor densities in drug naive SZ and in PD, as compared to normals (Rinne et al., 1990b; Sedvall et al., 1992; Shinotoh et al., 1993). In theory, the lack of agonist stimulation in PD is expected to increase the proportion of D_1 receptors in their high-affinity agonist state, as previously reported in the chronic reserpine-treated rat model (Rubinstein et al., 1990), while excess of DA in SZ would produce the contrary (Mamelak et al., 1993). This underscores the importance of developing D_1 agonist radioligands for imaging D_1 receptors in living human brains with PET.

The benzazepine *R/S*-SKF 82957 (*R/S*-(±)-3-methyl-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) displays agonistic activity for AC (EC_{50} = 0.6 μM) (Pfeiffer et al., 1982), and binds with high affinity and selectivity to the high-affinity sites of D_1 receptors (Ki = 0.9 nM) (Neumeyer et al., 1991). The synthesis, autoradiographic and initial *in vivo* evaluation in rats of the first selective D_1 agonist PET radioligand *R/S*-[^{11}C]SKF 82957 was recently reported (DaSilva et al., 1996a; DaSilva et al., 1996b). With the aim of increasing the signal-to-noise (specific to non-specific) ratios, we undertook the synthesis and *in vivo* characterization of the active enantiomer *R*(+)-SKF 82957 (Figure 1), labeled with ^{11}C. We report here its synthesis, biodistribution, competition and metabolism studies in rats, as well as its dosimetry estimates for human studies.

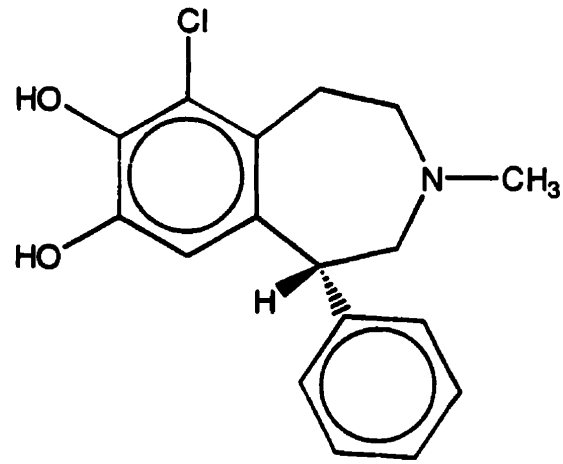

Fig. 1. Structure of *R*-SKF 82957.

2.3 MATERIALS AND METHODS

General

N-Ethyldiisopropylamine (Lancaster Synthesis Inc., NH, U.S.) was diluted in dimethylformamide (DMF) (100 mg/mL solution). *R/S*-SKF 81297•HCl and *R/S*-SKF 82957•HCl were generous gifts from SmithKline Beecham Pharm. (King of Prussia, PA, U.S.). *R*(+)-SKF 81297•HBr and *R*(+)-SKF 82957•HBr were purchased from Research Biochemicals Int. (RBI, MA, U.S.). DMF was stirred overnight with BaO, then distilled under reduced pressure from BaO and stored over 4 Å molecular sieves. Racemic [^{11}C]SKF 82957 was synthesized as described previously (DaSilva et al., 1996a). Semi-preparative and analytical HPLC in *R*-[^{11}C]SKF 82957 preparation were performed as previously reported for *R/S*-[^{11}C]SKF 82957 (DaSilva et al., 1996a). Thin-layer chromatographic (TLC) analyses were carried out on plastic-backed silica gel plates (60A K6F, Merck) with methanol/triethylamine 95/5 as the eluting solvent mixture. This system is capable of separating the nor-methyl SKF 81297 (R_f ~0.55) from SKF 82957 (R_f ~0.7). An automated TLC-Linear Tracemaster-20 analyzer (Berthold) was used to analyze the radioactive compounds on the TLC.

The following drugs were prepared in isotonic sterile solutions at pH 4.5-6.5 and injected at 1 mL/kg. *R*-SCH 23390•HCl (RBI) was dissolved in 0.9% saline. (-)-Sulpiride•HCl (Sigma Chem. Co., MO, U.S.) was dissolved in warm 4% ethanol/saline. *R/S*-SKF 82957•HCl was prepared for injection by dissolution in ethanol/1,2-propanediol/saline 5/12.5/82.5. Ritanserin•HCl (RBI) was dissolved in ethanol/1,2-propanediol/saline 5/20/75.

Synthesis of R-[^{11}C]SKF 82957

R-[^{11}C]SKF 82957 was prepared using the same conditions as *R/S*-[^{11}C]SKF 82957 (DaSilva et al., 1996a). Briefly, [^{11}C]methyl iodide, produced from [^{11}C]CO_2, was trapped in a 1 mL reacti-vial containing the desmethyl derivative *R*-SKF 81297•HBr (1 mg) in the presence of N-ethyldiisopropylamine (1.9 equivalents, 10% v/v solution in DMF) and DMF (185 μL) at –20 to -40°C. After 5 min at 85°C, *R*-[^{11}C]SKF 92057 was purified by semi-preparative HPLC. The resulting sterile and pyrogen free *R*-[^{11}C]SKF 82957 formulation (pH 6-7.5) was found, by analytical HPLC, to be stable to radiolysis for at least 90 min. Identity of the radioactive product as *R*-[^{11}C]SKF 82957 was determined by co-injection of authentic *R*-SKF 82957 using HPLC. In addition, TLC of the radioactive formulation showed one peak with the same R_f as co-spotted authentic *R*-SKF 82957.

In Vivo Binding Studies

Animal experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care and with approval from the Animal Care Committee at the Clarke Institute of Psychiatry. Rats were maintained on a 12 hour light/dark cycle with food and water available *ad libitum*. Except for the whole body distribution study, *in vivo* studies were done in a manner similar to that previously reported (DaSilva et al., 1996b) in male Sprague-Dawley rats (Charles River, Montreal, Canada) weighing 190-250 g.

TIME COURSE. Rats were injected with ~44 MBq (~1.2 mCi) of high specific activity *R*-[^{11}C]SKF 82957 (>18.5 GBq/μmol or >500 mCi/μmol, at time of injection). Radioactivity levels in different brain regions (see Fig. 2 A and B for list) and blood are expressed as percent injected dose per gram (%ID/g) of tissue.

COMPETITION STUDIES. Competition studies were carried out by either pre-treatment with sulpiride (5 mg/kg, i.v., 5 min prior to radioligand injection) (Hatano et al., 1989) or ritanserin (2.5 mg/kg, s.c., 100 min prior) (Leysen et al., 1985), followed by a tail vein injection (as above) of *R*-[^{11}C]SKF 82957; or using i.v. co-injection of either SCH 23390 (1.5 mg/kg) (Hatano et al., 1989; Sedvall et al., 1991) or *R/S*-SKF 82957 (10 mg/kg) (DaSilva et al., 1996b), together with *R*-[^{11}C]SKF 82957 *via* a tail vein. The animals were killed at 45 min after radiotracer administration, and the regional brain and blood distribution was carried out as above. All brain region values were added to give the mean %ID/g for the whole brain following each treatment.

WHOLE BODY STUDIES. Whole body distribution studies were performed in Sprague-Dawley rats (2 females and 2 males per time point). Animals were administered with *R/S*-[^{11}C]SKF 82957 *via* a tail vein (as above), then sacrificed by decapitation at 5, 15, 30 and 60 min post-injection. A blood sample was collected and whole tissues were dissected out (See Table 2 for list), counted (decay-corrected) in a gamma-counter (Cobra II, Canberra Packard), along with aliquots of the injected solutions as standards, and then weighed. Radioactivity remaining in the syringes and carcass was measured in a dose calibrator (CRC-712M, Capintec), and taken into account in the calculation of the injected dose. Data were calculated as % injected dose per organ (%ID).

STATISTICAL ANALYSIS. Statistical analysis was carried out using one-way ANOVA followed by Bonferroni's post-hoc comparisons tests. Since no significant difference was found in the regional brain retention of radioactivity among the different control groups with *R*-[^{11}C]SKF 82957 (at 45 min post-injection), the data from the controls

were pooled (n = 8) and used in statistical calculations. P values <0.05 were considered significant.

Dosimetry Calculations

The biodistribution of *R/S*-[^{11}C]SKF 82957 in rats was used to calculate the expected human dosimetry using the MIRD methodology (Loevinger et al., 1988). The percent injected dose per organ of the rat was converted to percent injected dose per human organ for the brain, liver, spleen, lungs and kidneys. The conversion factor relates the ratios of organ to body weights in the rat and human. The contents of the small and large intestine were added together and measured. Because of the short physical half-life of carbon-11 compared to the mean rate of transport through the human small intestine (Eve, 1966), the total radioactivity in the GI tract was assigned to the small instestine in our calculations. The penis on male rats was tied off and urine was collected directly from the bladder. The other organs listed in Table 2 were examined to rule out unexpected high uptake by those organs but the data were not used in the final dosimetry calculations. The estimated radiation dose was calculated with the MIRDOSE 3.1 software (Stabin, 1996).

Metabolism Studies

The metabolism of *R*-[^{11}C]SKF 82957 was examined in plasma and brain homogenates of a male Sprague-Dawley rat (300 g), 30 min after an injection of ~14.8 MBq (~4 mCi) *via* a tail vein (as above). Upon decapitation the rat brain was rapidly removed and stored on ice, and blood from the trunk was collected in a heparinized tube. This procedure

was repeated in another rat with a separate *R*-[^{11}C]SKF 82957 formulation in order to verify the reproducibility of the results.

PLASMA. Blood was centrifuged (1000 g, 5 min), and the resulting plasma (1 mL) was mixed with acetic acid 1% in water (3 mL) containing *R/S*-SKF 82957 (20 μg, as internal standard). The solution was passed through a preactivated (ethanol (10 mL) followed by acetic acid 1% (20 mL)) C_{18} Sep Pak Plus (Waters Co.). Acetic acid 1% (4 ml) was then passed through the column (twice) to ensure elution of polar hydrophilic metabolites. The hydrophobic fraction was eluted with ethanol 95%/glacial acetic acid 90/10 (4 mL). All eluted fractions, whole blood, plasma samples, and the C_{18} Sep Pak contents were counted for radioactivity in the gamma-counter. Recoveries of radioactivity were better than 97%. The organic fraction was then prepared for analysis by evaporation to dryness under vacuum, re-suspended in ~100 μL of the elution solvent, spotted onto the TLC plates, developed, and then analyzed using both the radioactivity scanner and ultraviolet absorption (254 nm). Control experiments were carried out with rat blood and ~7.4 MBq (~200 μCi) of authentic *R*-[^{11}C]SKF 82957 to validate the procedure.

Protein binding of *R*-[^{11}C]SKF 82957 to plasma was assessed *via* ultrafiltration centrifugation utilizing the Centrifree (Amicon, Beverly MA) kit. Plasma containing *R*-[^{11}C]SKF 82957 was centrifuged (1000 g, 45 min) at ambient temperature, and the resulting filtrate (plasma free fraction) together with a sample of plasma was counted in the gamma-counter.

BRAIN. A control rat was also killed at 30 min post-injection of saline into the lateral tail vein, and its brain removed. Both brains, the one from the rat injected with *R*-

[^{11}C]SKF 82957 and the control rat, were homogenized (Polytron) in ice-cold ethanol 95%/water 80/20 (10 mL) containing *R/S*-SKF 82957 (40 μg, as an internal standard). To the control rat brain mixture, ~7.4 MBq (~200 μCi) of *R*-[^{11}C]SKF 82957 was also added. Both homogenates were then centrifuged (82,000 g, 15 min). Glacial acetic acid (1 ml) was added to the resulting supernatant and evaporated to dryness. Then it was analyzed with the radioactivity scanner and UV (as above).

2.4 RESULTS

Radiochemistry

R-[^{11}C]SKF 82957 was synthesized by N-[^{11}C]methylation of the full D_1 agonist *R*-SKF 81297•HBr with [^{11}C]methyl iodide in the presence of N-ethyldiisopropylamine. Using the same conditions as for *R/S*-[^{11}C]SKF 82957, *R*-[^{11}C]SKF 82957 was prepared in high radiochemical yields 45-75% (decay-corrected, based on [^{11}C]CH_3I), purity (>99%) and specific activity (>37 GBq/μmol or >1000 mCi/μmol, at end-of-synthesis), in a synthesis time of 35 min (including quality control assays).

Regional Brain Distribution Studies

The time-activity curves of regional rat brain distribution of radioactivity following *R*-[^{11}C]SKF 82957 injection are presented in Figure 2 (A and B). High retention of radioactivity was found in the D_1 receptor-rich striatum and olfactory tubercles, while the lowest levels were obtained in the cerebellum, a region relatively devoid of D_1 receptors (Boyson et al., 1986; Savasta et al., 1986). All other brain regions exhibited radioactivity uptake and washout rates that paralleled that of the cerebellum but with slightly higher values. High striatum- and olfactory tublercles-to-cerebellum (signal-to-noise) ratios vs. time

were observed (Fig. 2 C), and reached 11.3 ± 1.9 and 9.7 ± 1.5, respectively, at 45 min post-injection. Other region-to-cerebellum ratios (e.g. frontal cortex) were approximately 2 throughout the study (Fig. 2 C). Compared to *R/S*-[^{11}C]SKF 82957, *R*-[^{11}C]SKF 82957 showed higher accumulation of radioactivity in the striatum and olfactory tubercles (~1.7 times), thereby improving the tissue contrast (Table 1).

The effects of pre-treatment or co-injection of various drugs on the regional brain distribution of *R*-[^{11}C]SKF 82957 in rats are depicted in Fig. 3. Co-administration of unlabeled *R/S*-SKF 82957 and SCH 23390 significantly reduced the retention of radioactivity in all brain regions to cerebellar levels. In contrast, no effect was observed in the radioactivity levels in any brain areas following treatment with high doses of the D_2 antagonist sulpiride or 5-HT_2 antagonist ritanserin. Whole brain uptake was significantly decreased only in blocking studies using D_1 competitors. These results suggest that *R*-[^{11}C]SKF 82957 binds selectively to D_1 receptors over D_2 and 5-HT_2 receptors.

Whole Body Distribution and Dosimetry Studies

The biodistribution of *R/S*-[^{11}C]SKF 82957 is given in Table 2. The main pathway of excretion is through the hepatobiliary route (%ID: Liver 4.2±5.83, GI content 62.0±15.1 after 1 hour) and significantly less through the urinary route (3.76 %ID at one hour). Gradual washout of activity from the brain is observed with an initial value of 1.04 ± 0.34 %ID at 5 min post-injection and 0.28 ± 0.08 %ID at one hour post-injection. These data were used to estimate the human dosimetry of *R/S*-[^{11}C]SKF 82957 which is summarized in Table 3. Because of the high rate of hepatobiliary excretion, the small intestine is the critical organ with 0.042 mGy/MBq (0.16 rad/mCi). Since the biodistribution of *R*-[^{11}C]SKF 82957 is

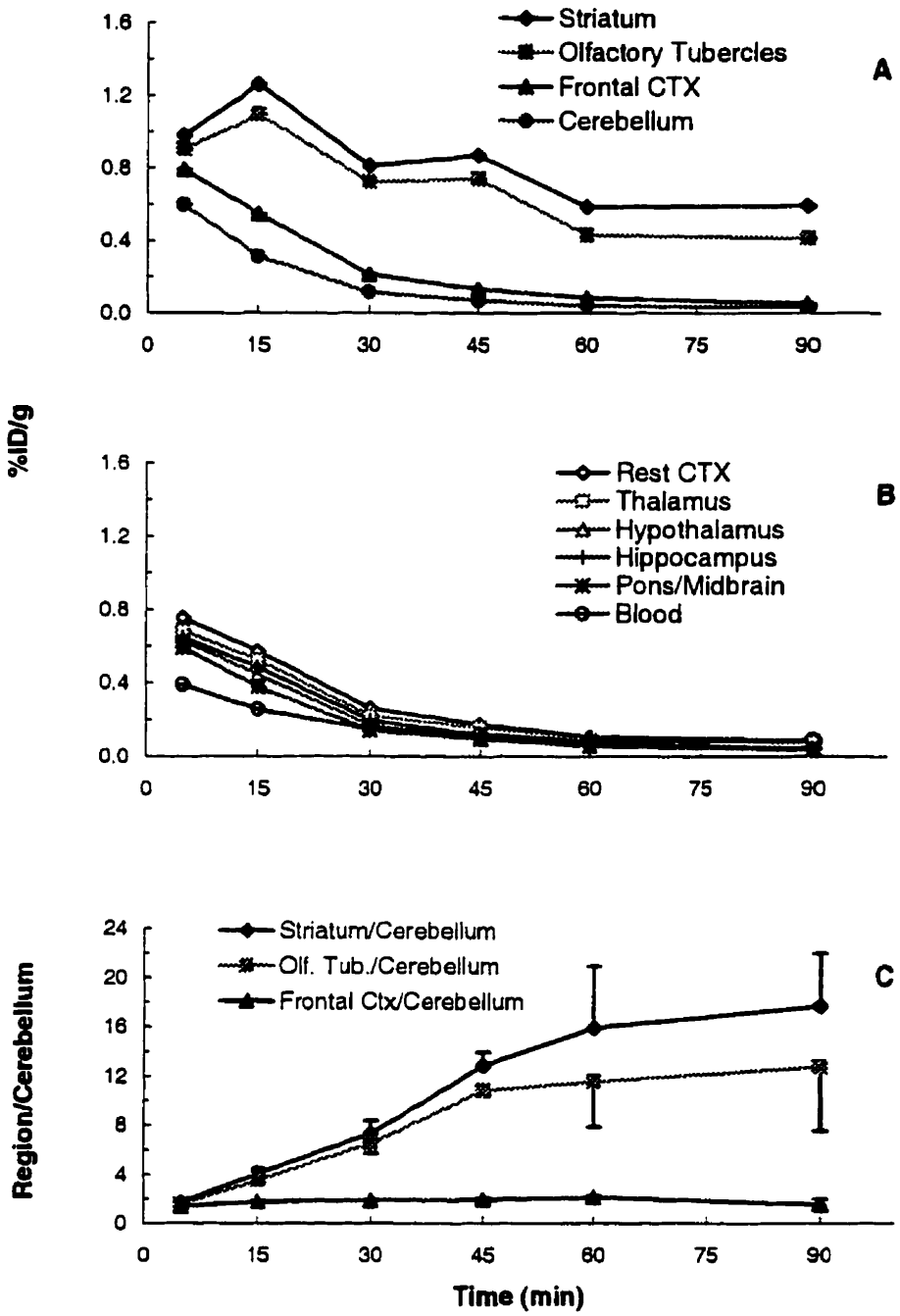

Fig. 2. (A and B) Regional brain uptake and blood distribution, and (C) region-to-cerebellum ratios (for striatum, olfactory tubercles and frontal cortex) of radioactivity in rats, following injection of R-[11C]SKF 82957 in rats. Data (A and B) are expressed as means of % injected dose per gram of tissue ± S.D. (n = 4).
CTX: cortex; Olf.: olfactory; Tub.: tubercles.

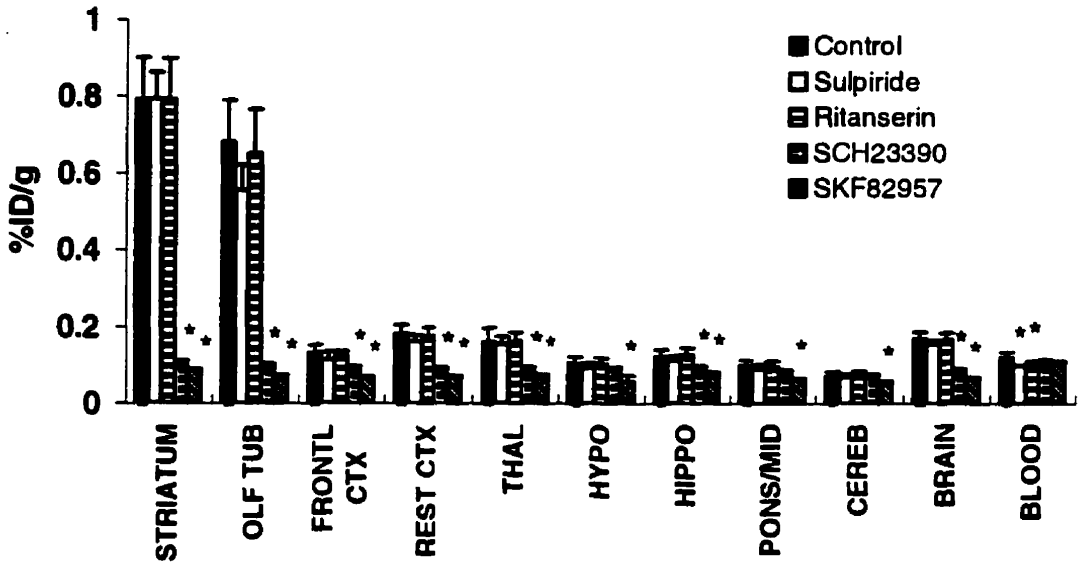

Fig. 3. Effect of treatment with various drugs on the regional, blood and whole brain retention of radioactivity in rats, 45 min R-[11C]SKF 82957 postinjection. Data are expressed as means of % injected dose per gram of tissue ± S.D. (n = 8 for controls and n = 5 for drug treatment groups). OLF: olfactory; TUB: tubercles; CTX: cortex; THAL: thalamus; HYPO: hypothalamus; HIPPO: hippocampus; CEREB: cerebellum. * $p < 0.05$ (ANOVA with bonferronni's post-hoc test).

TABLE 1. Biodistribution of R-[^{11}C]SKF 82957 in Rats, 45 min after Administration†

Brain Region/Organ	R/S-[^{11}C]SKF 82957††	R-[^{11}C]SKF 82957
Striatum	0.47 ± 0.11*	0.79 ± 0.11*
Olfactory Tubercles	0.38 ± 0.10*	0.67 ± 0.11*
Frontal Cortex	-------	0.13 ± 0.02
Rest of Cortex	-------	0.18 ± 0.02*
Thalamus	0.17 ± 0.05*	0.16 ± 0.03
Hypothalamus	0.16 ± 0.04*	0.10 ± 0.02
Hippocampus	0.14 ± 0.04*	0.12 ± 0.02
Pons/Midbrain		0.10 ± 0.01
Cerebellum	0.08 ± 0.03	0.07 ± 0.01
Brain	0.16 ± 0.04	0.17 ± 0.02
Lung	0.30 ± 0.10	0.29 ± 0.05
Heart	0.08 ± 0.02	0.08 ± 0.01
Liver	0.70 ± 0.20	0.93 ± 0.14
Blood	0.09 ± 0.02	0.12 ± 0.01

† Mean of %ID/g ± S.D. of control rats (n=24 for R/S-[^{11}C]SKF 82957, n = 8 for R-[^{11}C]SKF 82957).

†† Values from Dasilva et al., 1996b.

* $p < 0.05$ compared to cerebellum.

TABLE 2. Biodistribution of R/S-[^{11}C]SKF 82957 in rats.*

Organ or Tissue	Time (min) 5	15	30	60
Pituitary	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
Eyes	0.03 ± 0.01	0.03 ± 0.00	0.02 ± 0.01	0.01 ± 0.00
Ascending/transverse LI†	0.36 ± 0.14	0.30 ± 0.04	0.25 ± 0.08	0.13 ± 0.03
Descending LI†	0.12 ± 0.05	0.10 ± 0.02	0.06 ± 0.02	0.05 ± 0.02
Small intestine	3.37 ± 1.05	9.72 ± 2.91	6.88 ± 3.70	8.86 ± 5.83
Testicles	0.64 ± 0.10	0.64 ± 0.00	0.54 ± 0.03	0.19 ± 0.05
Ovaries	0.13 ± 0.02	0.08 ± 0.01	0.04 ± 0.01	0.03 ± 0.00
Heart wall	0.48 ± 0.10	0.23 ± 0.03	0.17 ± 0.10	0.05 ± 0.01
Lungs	2.52 ± 0.59	1.15 ± 0.15	0.90 ± 0.56	0.22 ± 0.08
Spleen	0.45 ± 0.06	0.33 ± 0.07	0.24 ± 0.14	0.08 ± 0.02
Adrenals	0.07 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.01 ± 0.01
Stomach	0.60 ± 0.32	0.73 ± 0.52	0.56 ± 0.44	0.16 ± 0.07
Urinary bladder wall	0.03 ± 0.01	0.03 ± 0.01	0.07 ± 0.09	0.11 ± 0.16
Brain	1.04 ± 0.34	0.92 ± 0.14	0.60 ± 0.15	0.28 ± 0.08
Urine	0.74 ± 0.23	0.13 ± N/A	1.42 ± N/A	3.76 ± N/A
GI contents†	7.70 ± 4.31	20.84 ± 4.08	39.64 ± 8.44	62.0 ± 15.13
Liver	9.19 ± 2.44	8.83 ± 1.21	7.24 ± 2.51	4.20 ± 0.46
Kidneys	3.65 ± 0.71	2.18 ± 0.42	1.60 ± 0.94	0.76 ± 0.12

* Data are expressed as % injected dose per organ ± SD.

† LI = Large intestine; GI = Gastro-intestinal.

TABLE 3. Calculated Absorbed Dose to Human for *R/S*-[^{11}C]SKF 82957 Based on Biodistribution in Rats

Organ	rad/mCi
Whole Body	0.005
Red Marrow	0.003
Ovaries	0.003
Testes	0.003
Small Intestine	0.157
Brain	0.004
Heart Wall	0.003
Kidneys	0.014
Liver	0.022
Lungs	0.012
Spleen	0.004

similar to that of the racemic compound, its dosimetry is expected to be similar.

Metabolism Studies

At 30 min post-injection of *R*-[^{11}C]SKF 82957, ~12% of the total radioactivity in plasma was present as polar metabolites in the aqueous fractions and ~86% in the ethanol eluant, following solid-phase extraction. Radioactivity analysis of the organic fraction revealed only the presence of unmetabolized *R*-[^{11}C]SKF 82957. This single peak had the same Rf as that of the co-eluted *R/S*-SKF 82957 (as detected by UV) and that obtained in the control experiment using authentic *R*-[^{11}C]SKF 82957. Both TLC analyses of the homogenized brain extracts from the injected and control rats indicated only the presence of unchanged *R*-[^{11}C]SKF 82957. The plasma protein binding fraction was found to be approximately 93%.

2.5 DISCUSSION

In vitro assays involve homogenization of postmortem tissue samples, or preparation of an autoradiographic slice in the presence of a radioligand, usually labeled with a long half-life radioisotope such as tritium, and incubation in a physiological buffer. These *in vitro* experiments may be unable to reproduce reliably *in vivo* conditions, especially those involving the complex mechanisms involved in the high- and low-affinity states of D_1 receptors coupled to G-proteins. It is thus important to determine the *in vivo* pharmacological profile of a D_1 agonist PET radioligand.

The pure enantiomer *R*-[^{11}C]SKF 82957 was recently developed due to the availability of the precursor *R*-SKF 81297. Using the same conditions as for *R/S*-[^{11}C]SKF 82957, we have prepared *R*-[^{11}C]SKF 82957 in higher yields (45-75%, compared to 20-45% for *R/S*-[^{11}C]SKF 82957 (DaSilva et al., 1996a). *In vivo* evaluation of *R*-[^{11}C]SKF 82957 in rats revealed a regional brain distribution consistent with those previously reported for selective D_1 receptor radioligands (Boyson et al., 1986; Dubois et al., 1986; Savasta et al., 1986). Compared to *R/S*-[^{11}C]SKF 82957 (DaSilva et al., 1996b), *R*-[^{11}C]SKF 82957 showed higher retention of radioactivity in the striatum and olfactory tubercles (~1.7 times), thereby improving the specific-to-non-specific binding ratios. Consequently, higher signal-to-noise ratios are obtained with the *R*-enantiomer of [^{11}C]SKF 82957 as compared to the racemic mixture. These results are in agreement with previous studies which demonstrated that the *R*-(+) enantiomer of substituted 1-phenyl-3-benzazepines bind with higher affinity and selectivity to D_1 receptors in comparison to the S-(-) enantiomer, and that agonistic activity for AC resided almost exclusively in the *R*- antipode (Kaiser et al., 1982; Neumeyer et al., 1992). *R*-[^{11}C]SKF 82957 uptake was reduced uniformly across the brain regions to cerebellum levels, only in studies using D_1 competitors (SCH 23390 and SKF 82957), indicating the presence of specific binding to D_1 receptors. In contrast, pretreatment with high doses of the D_2 antagonist sulpiride or 5-HT_2 antagonist ritanserin showed no effect on *R*-[^{11}C]SKF 82957 binding as compared to controls, suggesting high binding selectivity for D_1 receptors.

Whole body distribution studies of *R/S*-[^{11}C]SKF 82957 in rats revealed that most of the radioactivity was excreted by the hepatobiliary route. Dosimetry calculations indicated that the small intestine is the limiting organ (0.16 rad/mCi). Similar dosimetry is expected

for *R*-[^{11}C]SKF 82957, since it displays comparable biodistribution. One major limitation of extrapolating the rat data to humans is the potential difference in the hepatic clearance rate of SKF 82597 in the two species. Human hepatic clearance rate of [^{11}C]SKF 82957 will be required to refine the dosimetry estimates. Plasma radioactivity analysis revealed ~86% unchanged *R*-[^{11}C]SKF 82957 at 30 min post-injection in the rat. Only unmetabolized *R*-[^{11}C]SKF 82957 was present in the rat brain extracts.

In conclusion, the results of this study demonstrate that *R*-[^{11}C]SKF 82957 has a high binding selectivity for D_1 receptors, acceptable radiation dosimetry, and no metabolites in rat brain extracts. As expected, the active *R*-[^{11}C]SKF 82957 increased the signal-to-noise ratios as compared to the D_1 agonist *R/S*-[^{11}C]SKF 82957, allowing more sensitive *in vivo* measurements of D_1 receptors.

2.6 STATEMENT OF SIGNIFICANCE

The work presented in this paper demonstrates that our C_{18} Sep Pak elution and TLC methods are capable of determining the metabolic profile of R-[^{11}C]SKF 82957 in rats following intravenous administration. The results indicate that most of the radioactivity is excreted by the hepatobiliary route, and that there is a low presence of metabolites in plasma. The hydrophilic metabolites, corresponding to <15% of total plasma radioactivity at 30 min post-injection, are unlikely to cross the BBB.

3.0 Human Brain Imaging with the Dopamine D_1 Agonist R-[^{11}C]SKF 82957 and Positron Emission Tomography

Jean N. DaSilva, Robert A. Schwartz, Douglas Hussey, Kevin Cheung,

Alan A. Wilson, and Sylvain Houle

To be submitted: *Neuro Report*

The experiment described in this paper requires the assistance of at least five people in order to be carried out successfully. I therefore assisted in the performance of all studies described herein and in the preparation of this manuscript. **The chemistry, PET imaging, ROI drawing and the kinetic modeling to achieve the binding potential was not conducted by myself and will therefore not be discussed outside of this chapter.**

3.1 ABSTRACT

Alterations have been reported in the affinity and in the proportion of the high-affinity sites of dopamine D_1 receptors in neuropsychiatric disorders in comparison to controls. Only D_1 agonist radioligands are capable, in theory, of selectively assessing the high-affinity state of D_1 receptors. We report here the first PET imaging trials of the D_1 agonist R-[^{11}C]SKF 82957 in healthy human subjects. Radioactivity accumulation was detected in the striatum, and to a lesser extent in the cortex. A significant correlation in the striatal binding potential values, with a decline of ~0.9% per year, was obtained using the Lammertsma and Logan methods. Test-retest results indicate good reproducibility of the striatal binding potential. High protein binding and radioactivity levels are found in the plasma reaching steady-state levels after 5 min post-injection. Analysis of metabolites in human plasma revealed the presence of >85% unchanged radioligand up to 80 min post-injection. Thus R-[^{11}C]SKF 82957 has good potential as a ligand for human brain PET imaging.

3.2 INTRODUCTION

Dopamine D_1 receptors exist in either the high- or low-affinity state for agonists or partial agonist binding. Antagonists do not differentiate between these states. The binding of a D_1 agonist to the functional high-affinity state of D_1 receptors (D_1^{High}) coupled to a stimulatory G-protein (Gs), activates adenylyl cyclase leading to the formation of cAMP (Kimura et al., 1995; Rubinstein et al., 1990; Seeman and Grigoriadis, 1987). Only D_1 agonist radiotracers are thus capable of selectively assessing the *in vivo* density of D_1^{High} receptors using positron emission tomography (PET). Dopamine D_1 receptors are more abundant than D_2 receptors in human striatum (Seeman et al., 1987), and both receptors are primary targets for drugs used to treat many psychomotor disorders (Clark and White, 1987; Needham et al., 1993; Waddington and O'Boyle, 1989).

All D_1 radiotracers that have previously been developed for PET or single photon emission tomography (SPECT) are D_1 receptor antagonists, and thus bind to the total density of D_1 receptors. No difference in D_1 receptor densities was found in the striatum of drug naive schizophrenics or parkinsonians, relative to normals, using the D_1 antagonist [^{11}C]SCH 23390 and PET (Rinne et al., 1990b; Sedvall et al., 1992). Interestingly, Okubo et al (1997) has recently reported a reduction in the binding of [^{11}C]SCH 23390 in the prefrontal cortex. In theory, the lack of agonist stimulation in Parkinson's disease is expected to increase the proportion of D_1 receptors in their high-affinity agonist state in the caudate-putamen, as previously reported in the chronic reserpine-treated rat model (Rubinstein et al., 1990), while excess of dopamine in schizophrenia would produce the contrary (Mamelak et al., 1993). These findings underscore the importance of developing D_1 agonist PET radioligands for

imaging D_1 receptors in different neurological and psychiatric disorders and in drug dependence.

R-SKF 82597 (R(+)-6-chloro-7,8-dihydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine), labeled with the positron-emitting isotope carbon-11, was recently reported to display higher striatal binding (~1.7 times) *in vivo* in rats than R/S-[^{11}C]SKF 82957 (Ki D_1^{High} = 0.9 nM (Neumeyer et al., 1991); adenylyl cyclase EC_{50} = 0.6 μM (Pfeiffer et al., 1982; DaSilva et al., 1999a)). This paper presents the first PET images in the living human brain using a direct dopamine agonist radioligand.

3.3 MATERIAL AND METHODS

PET imaging

This study was approved by the University of Toronto Human Subjects Review Committee. Eleven healthy (8 males, 3 females) volunteers (age-range 23-42 years) participated in this study after giving written informed consent. They were free of general medical illness and medication, and had no history of head injury, alcohol or drug abuse. Prior to the PET study, a stereoadaptor head holder (Sandström Trade and Technology Inc., Canada) was adjusted for each subject, and secured to the scanning gantry to minimize head motion during acquisition. A 10 minute transmission scan was acquired with a rotating ^{68}Ge pin source for subsequent attenuation correction of the emission scans. R-[^{11}C]SKF 82957 (DaSilva et al., 1999a) was injected intravenously (~9.1 mCi in 10 mL, using a PHD2000 Harvard Apparatus syringe pump at 30 mL/min) in tracer dose (specific activity 1400 ± 780 Ci/mmol, corresponds to 1.3 - 4.5 μg of the free base product per injection). Sequential

images of the brain (one image per min for the first 15 min, then 15 x 5 min frames, for a total of 30 frames) were obtained over a 90 min period following injection, with a GEMS PC2048-15B brain PET scanner (15 slices, resolution 4.5 x 4.5 x 4.5 mm FWHM in air, with 6.5 mm inter-slice separation). Following acquisition, the images were corrected for attenuation and reconstructed by filtered back-projection (Hann filter, 5 mm) (Guttman et al., 1997; Houle et al., 1997).

Image analysis

Magnetic Resonance Imaging (MRI) scans were acquired for each subject in order to provide anatomical landmarks for region of interest (ROI) definition (GEMS Signa 1.5 Tesla scanner spin-echo sequence T_2-weighed image). Contiguous 3mm thick T_2-weighed slices were obtained for coregistration with the PET images. The MRI slices were aligned to the PET data using the ANALYZE (CNS Software, Rochester, MN, USA) package which implements an automated algorithm.

Paired left and right ROIs were drawn on the MRI images following coregistration. The regions include the striatum, thalamus, prefrontal cortex and cerebellum drawn on two contiguous slices. The corresponding regions on the two slices were averaged to avoid errors due to minor head tilt, and to improve the signal and statistics. These ROIs were transferred to the corresponding PET images, and decay-corrected time-activity curves were obtained for each ROI.

The binding potential (BP) of *R*-[^{11}C]SKF 82957 was calculated by both the Lammertsma's simplified reference tissue method (Lammertsma and Hume, 1996) and the Logan's graphical analyses methods (Logan et al., 1996). These models allow for

quantification of receptor kinetics without measuring the arterial input function, thus avoiding invasive arterial cannulation.

R-[^{11}C]SKF 82957 scanning reproducibility

Six of the subjects received a second scan 14-104 days apart, in order to validate the reproducibility of the experimental procedures on two occasions (test-retest study), including a new synthesis, ROI drawing and data analysis. Having two raters analyse the data from the same six scans assessed the reliability of our PET technique, providing a measure of the inter-rater reliability. Intra-rater reliability was determined by having the same rater evaluate the same PET scan on two separate occasions.

Statistical analysis

Left and right striatum BP values calculated using the Lammertsma and Logan methods were averaged, since no significant difference was observed by Wilcoxon signed rank test. Linear regression analysis was used to test for age effect on the BP, and for the correlation analyses between the BP values obtained by the Lammertsma and Logan methods. Scan reproducibility was measured by ANOVA. Inter- and intra-rater reliability was assessed via the intraclass correlation coefficient type III (Shrout and Fleiss, 1979). This provides a value between 0 (no reliability) and 1 (perfect reliability). Analyses were carried out using SPSS software (SPSS Inc., IL, USA).

Fig. 1. R-[^{11}C]SKF 82957 PET image from a healthy subject, corresponding to the summed 0-90 min scan.

R-[^{11}C]SKF 82957 plasma analysis

Venous blood samples were withdrawn at 2, 5, 10, 20, 30, 45, 60 and 80 min post-injection from four subjects for metabolite analysis in the plasma, using the method published for rat blood (DaSilva et al., 1999a). Briefly, plasma (1 mL), mixed with acetic acid 1% in water, is passed through a preactivated C_{18} Sep Pak Plus (Waters Co.). Polar metabolites are eluted with the acetic acid 1% solution. The remaining hydrophobic fraction was eluted with ethanol 95%/glacial acetic acid 90/10, evaporated to dryness, and examined for radioactivity using thin-layer chromatography (TLC; 60A K6F, Merck; methanol/triethylamine 95/5) and a Berthold (Tracemaster-20) linear analyser, and by ultraviolet absorption (254 nm). Over 95% of radioactivity was recovered with this procedure at each time point. Plasma protein binding of R-[^{11}C]SKF 82957 was assessed via ultracentrifugation through Centrifree membrane filters (Amicon, Beverly, MA).

3.4 RESULTS

Higher radioactivity accumulation was observed in the D_1 receptor-rich caudate-putamen, and the confluence of the venous sinus (radioactivity in blood), as well as, to a lower extent, in the cortex (Figure 1). The corresponding decay-corrected time-activity curves of selected ROIs are depicted in Figure 2. The striatal uptake was rapid initially and peaked at ~10 min, followed by a gradual washout. The peak striatal uptake was 2.5 ± 0.4% of the injected dose per litre (%ID/L). It was higher than in the cerebellum (reference region devoid of significant amount of D_1 receptors (Cortés et al., 1989a)) throughout the scanning period. Time-activity curves of the thalamus and prefrontal cortex paralleled the cerebellum,

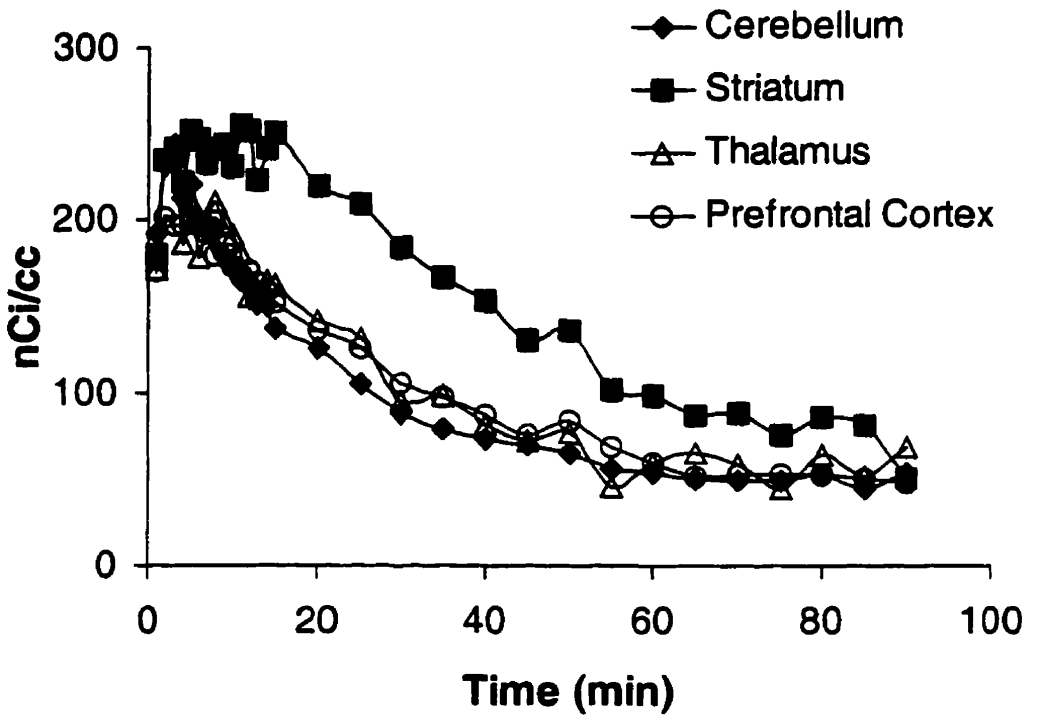

Fig. 2. Time-activity curves for regional radioactivity (nCi/mL) of a subject following intravenous injection of R-[^{11}C]SKF 82957.

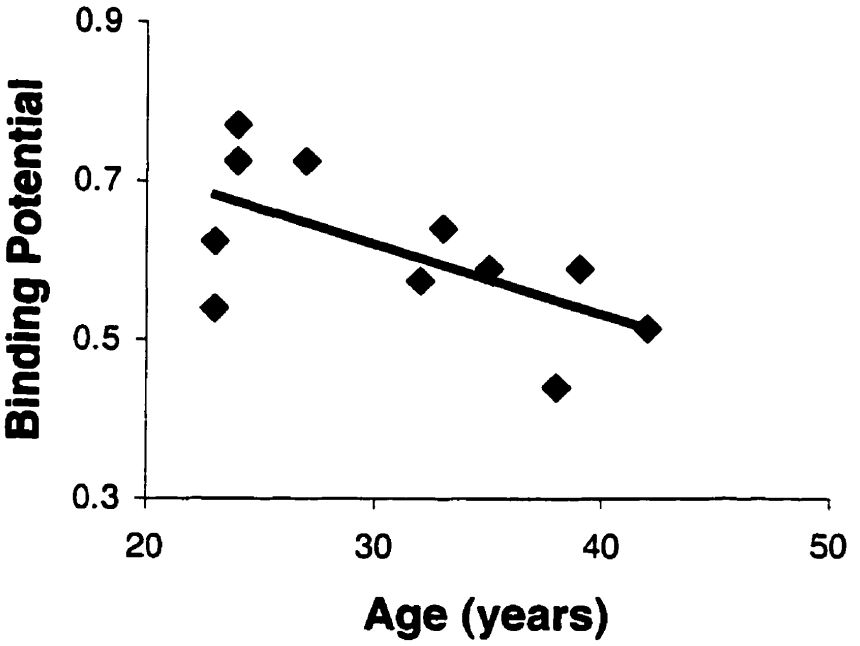

Fig. 3. Age-related decrease in the binding potential in the striatum as measured with the Lammertsma method for the R-[^{11}C]SKF 82957 study ($r = -0.65$, $p < 0.05$).

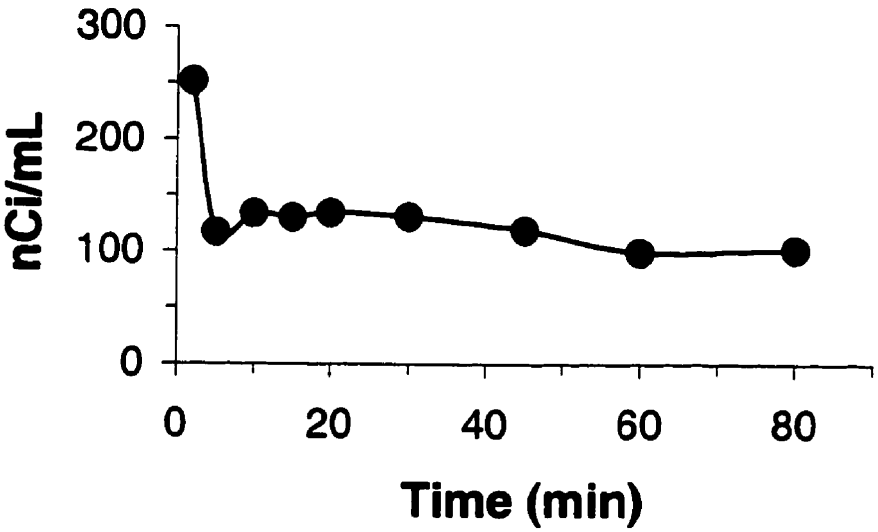

Fig. 4. Radioactivity (nCi/mL) in plasma after an intravenous injection of R-[^{11}C]SKF 82957 in one subject.

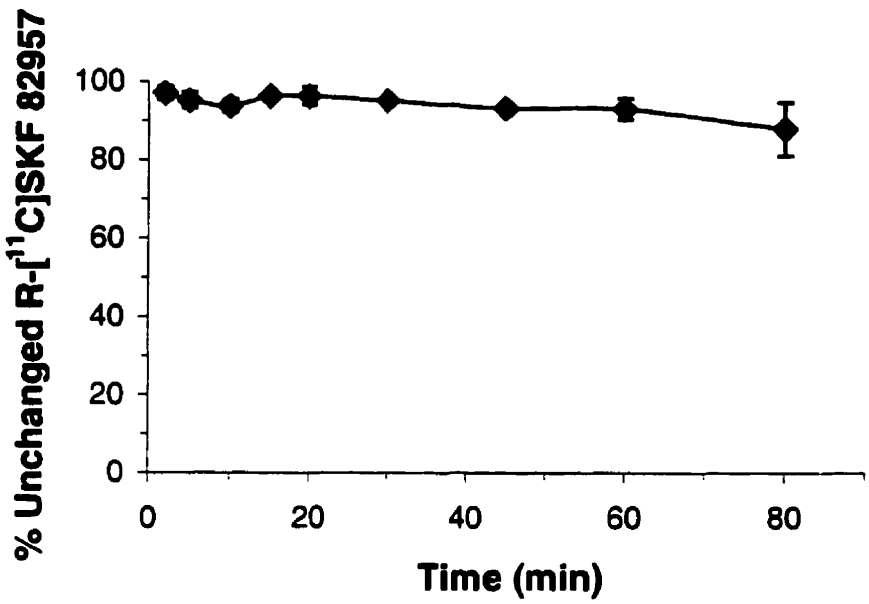

Fig. 5. Percentage of unchanged R-[^{11}C]SKF 82957 in human plasma as a function of time post-injection.

and were of slightly higher values (Fig 2). Therefore, it was not possible to accurately calculate the BP in the thalamus and prefrontal cortex. There is an excellent correlation in the BP values calculated by the Lammertsma and Logan methods in the striatum ($r = 0.99$, $p<0.001$). An age-related decrease of ~0.9% per year ($r = -0.65$, $p < 0.05$) was observed in R-[^{11}C]SKF 82957 striatal binding (Fig 3). No significant difference in the striatal BP was observed between scan one and scan two ($F_{1,11}$ $p=0.3$). The striatal inter-rater reliability was $r = 0.66$ with an intra-rater reliability of $r = 0.59$. Plasma radioactivity was high throughout the scanning period, reaching almost steady-state levels 5 min post-injection (Fig 4), with high protein binding (~97%). Using the C_{18} Sep Pak solid-phase column extraction system, analysis of plasma at 80 min post injection revealed <15% of total radioactivity in the aqueous fractions as polar metabolites, and >85% in the ethanol fraction (see Fig 5). Only the presence of unchanged R-[^{11}C]SKF 82957 was detected in the organic fraction by TLC .

3.5 DISCUSSION

In vivo animal studies have demonstrated that the D_1 agonist R-[^{11}C]SKF 82957 binds differently than the D_1 antagonist [^{11}C]SCH 23390 in the striatum of rats exhibiting D_1 receptor supersensitivity, following chronic treatment with SCH 23390 (Greenwald et al., 1999a) and reserpine (DaSilva et al., 1998a). These findings indicate that both radioligands bind to a different subpopulation of D_1 receptors. The present study is the first to use a direct agonist radioligand of the dopamine system in humans with PET. The high radioactivity retention observed in the caudate-putamen is likely due to D_1 receptors, since we have recently demonstrated that R-[^{11}C]SKF 82957 binds selectively *in vivo* to D_1 receptors as

opposed to D_2 and 5-HT_2 receptors in the rat striatum (DaSilva et al., 1999a). Racemic SKF 82957 was previously reported to bind only to D_1^{High} (Ki = 0.9 nM, Neumeyer et al. 1991; Ki SCH 23390 = 0.15 nM (Andersen and Gronvald, 1986)). However, most of the affinity for D_1 receptors is expected to come from the R-enantiomer of SKF 82957, since R-[^{11}C]SKF 82957 displays ~1.7 times higher *in vivo* binding in the D_1-rich striatum in rats in comparison to R/S-[^{11}C]SKF 82957 (DaSilva et al., 1999a). Furthermore, preceding studies have found that the high-affinity state represents 20-40% of the total density of D_1 receptors (De Keyser et al., 1988; Mamelak et al., 1993; Rubinstein et al., 1990; Sidhu et al., 1991). Therefore, a lower striatal BP is expected with R-[^{11}C]SKF 82957 in comparison to [^{11}C]SCH 23390.

Previous studies with the structurally similar benzazepine analog, [^{11}C]SCH 23390, revealed that the major metabolites were the conjugated *O*-sulphate and *O*-glucuronide polar derivatives (Swahn et al., 1992; Swahn et al., 1994). These hydrophilic metabolites are not likely to cross the blood-brain barrier (BBB). Contrary to [^{11}C]SCH 23390 (presence of 13% unchanged compound at 42 min postinjection (Swahn et al., 1992; 1994)), >85% unchanged R-[^{11}C]SKF 82957 was present in human plasma at 80 min postinjection with the rest being polar hydrophilic labeled metabolites unlikely to cross the BBB. In principle, the lower presence of metabolites in the plasma is beneficial, as it decreases the possibility of metabolites crossing the BBB and interfering with R-[^{11}C]SKF 82957 binding in the brain. Chromatographic analysis of rat brain extracts indicated the absence of labeled metabolites in brain tissue at 30 min post injection of R-[^{11}C]SKF 82957 (DaSilva et al., 1998b). High radioactivity levels were found in human plasma reaching steady-state levels after 5 min post-injection, indicating a slow excretion process from the blood. This finding is contrary to

what was observed in rats with plasma radioactivity levels decreasing progressively versus time, *via* excretion mainly through the hepatobiliary route (DaSilva et al., 1999a).

The excellent correlation in the striatal BP values obtained using the Lammertsma and Logan methods suggests that R-[^{11}C]SKF 82957 striatal BP can be calculated using either of these non-invasive reference tissue methods, avoiding the need for arterial blood sampling. The present study demonstrates a significant age-related loss of R-[^{11}C]SKF 82957 binding sites *in vivo* in humans, with ~0.9% loss per year of life (between 23-42 years). Decrease in striatal D_1 receptors (~0.67% loss per year) was also previously reported *in vivo* using [^{11}C]SCH 23390 and PET (Iyo and Yamasaki, 1993; Suhara et al., 1991), and in *in vitro* studies (Araki et al., 1997; Rinne et al., 1990a). Giorgi et al (1992) has shown that the reduction in D_1 receptor density in rat striatum was a result of a greater decrease in the receptor production as compared to the receptor degradation rate. A similar rate of decline (6-8% decrease per decade) of D_2 receptors and the dopamine transporters (DAT) was also found in humans (Antonini et al., 1993; Iyo and Yamasaki, 1993; Rinne et al., 1993; van Dyck et al., 1995; Volkow et al., 1996). This age-related decline in dopamine D_1 and D_2 receptors, and in the DAT may contribute to the decrease in the motor function observed with age.

The striatal test-retest data with R-[^{11}C]SKF 82957 indicates that similar BP values are obtained on the same person on two different occasions, therefore providing good reproducibility. The intraclass correlation coefficient was used as a measure of reliability of our raters to process the R-[^{11}C]SKF 82957 PET data from the same subject. The inter- and intra-rater reliability analyses indicate that R-[^{11}C]SKF 82957 can be used to accurately measure the striatal BP for a given ROI on two occasions with good reliability.

In conclusion, this study indicates that R-[^{11}C]SKF 82957 binds *in vivo* in the human caudate-putamen, and exhibits a rapid accumulation of radioactivity in the plasma throughout the scanning period, a low presence of metabolites, and good reproducibility and reliability. The striatal binding can be measured by the Lammertsma and Logan reference tissue methods. As seen with other post-synaptic dopamine PET radioligands, a decline in the BP is observed in the striatum between 23-42 years. These results suggest that R-[^{11}C]SKF 82957 is the first ligand to successfully be used to evaluate a G-protein-coupled dopamine receptor-agonist complex *in vivo* with PET in humans.

3.6 STATEMENT OF SIGNIFICANCE

The ultimate objective of this project is to utilize the selective D_1 receptor agonist R-[^{11}C]SKF 82957 to image both normal and neuropsychiatric subjects with PET. The results presented in this paper are those of the first human PET scans with R-[^{11}C]SKF 82957 on normal subjects. They indicate that this novel radioligand has good potential to be used as an *in vivo* marker to measure the functional high-affinity D_1 receptor with PET.

4.0 Effect of Chronic SKF 81297 Agonist and Unilateral 6-Hydroxydopamine Treatment on the In Vivo Binding of the D_1 Agonist R-[^{11}C]SKF 82957 in Rats

Robert A. Schwartz, Jean N. DaSilva, Paul J. Fletcher, Alan A. Wilson, and Sylvain Houle.

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The experiment described in this paper requires the assistance of at least five people in order to be carried out successfully. **Except for the chemistry, I performed all the experiments, including the data analysis, figures and writing of this manuscript.**

4.1 ABSTRACT

Supersensitization following experimental manipulation of receptor function has been well characterized in several treatment paradigms. One possible explanation for the enhanced agonist response is an increase in the proportion of receptors in the high-affinity state, with or without an increase in the total receptor density. Only agonists are able to characterize the functional high-affinity state, while antagonists cannot. Two different pharmacological treatments, previously shown to cause D_1 receptor supersensitivity in rats, were studied for changes in the binding of the D_1 antagonist SCH 23390 and the D_1 agonist R-SKF 82597, both labeled with carbon-11. Rats treated twice daily with the full D_1 agonist SKF 81297 for 21 days followed by a 7 day withdrawal period showed no significant difference in regional brain uptake or region-to-cerebellum ratios of either [^{11}C]SCH 23390 or R-[^{11}C]SKF 82957. Similarly, unilateral 6-hydroxydopamine lesioning, followed by apomorphine screening for contralateral rotation, failed to cause significant differences in the brain distribution of [^{11}C]SCH 23390 and R-[^{11}C]SKF 82957 in the lesioned versus the non-lesioned sides. These results suggest that the behavioral supersensitization induced by these treatments is due to changes at components of the signal transduction pathway beneath that of the D_1 receptor.

4.2 INTRODUCTION

Behavioural supersensitization is characterized as an enhanced response to an agonist following experimental manipulation of receptor function. The mechanism of this modified response is complex, and in many cases the exact physiological alterations which permit supersensitization are unclear. The dopaminergic receptor system, comprising of the D_1-like and D_2-like receptor families (Kebabian and Calne, 1979; Niznik and Van Tol, 1992) can be made supersensitive following diverse pharmacological treatments.

Supersensitization of D_1 receptors occurs following chronic direct or indirect agonist activation. DA receptor stimulation of rats previously treated chronically with cocaine leads to an increase in locomotor response (Post and Rose, 1976; Stripling and Ellinwood, 1977), which is associated with the D_1 receptor as it is blocked by D_1 antagonists (White et al., 1998). Similarly, chronic D_1 agonist treatment, followed by a specified period of withdrawal, has been reported to cause D_1 receptor supersensitization as observed in behavioral and electrophysiological studies (Hu et al., 1992; White et al., 1990).

Unilateral lesioning with the neurotoxin 6-hydroxydopamine (6-OHDA) has been widely employed in rats to destroy the dopaminergic projections from the substantia nigra pars compacta to the striatum, as an animal model of hemiparkinsonism (for review see (Kaakkola and Teravainen, 1990; Mokry, 1995). If this procedure is successful, these rats will initially exhibit a postural shift toward the ipsilateral lesioned side, and when challenged with the mixed D_1/D_2 agonist apomorphine will rotate in the contralateral direction (Hudson et al., 1993; Ungerstedt, 1971a). This contralateral rotation is also observed with the administration of selective D_1 receptor agonists as well (Arnt and Hyttel, 1984; Gnanalingham et al., 1995b; 1995c; Matsuda et al., 1992), indicating that this receptor has

become supersensitive. However, the mechanism of this measured sensitivity remains equivocal. Unilateral 6-OHDA lesions were previously reported to induce upregulation (Iwata et al., 1996; Porceddu et al., 1987), downregulation (Joyce, 1991), and no change (Graham et al., 1990; Lawler et al., 1995; Trugman et al., 1990) in striatal D_1 receptor density.

D_1 receptor supersensitization can arise due to an upregulation of receptor numbers (Bmax) and/or an increase in effector enzyme activity. Alternatively, an increase in the proportion of D_1 receptors in the high-affinity state (D_1^{HIGH}), with or without a change in the receptor Bmax could also account for the observed supersensitivity.

Previous *in vitro* studies have demonstrated that the G protein linked D_1 receptors exist in either a high- or a low-affinity binding state (Hess et al., 1986b; Seeman and Grigoriadis, 1987). The current ternary complex model of receptor dynamics states that agonist occupancy of D_1^{HIGH} receptors is related to biological response and that this conformation occurs when the receptor is functionally coupled to the G protein (De Lean et al., 1980; Mackay, 1990; Rodbell, 1980). Only agonists or partial agonists are capable of differentiating these receptor states, while antagonists cannot (De Lean et al., 1980; Kimura et al., 1995; Stadel et al., 1980). Therefore, in theory, only an agonist is able to quantify *in vivo* the differences in these states.

R/S-SKF 82957 ((±)-3-methyl-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) is an agonist (EC_{50}=0.6μM (cAMP) (Pfeiffer et al., 1982)), that binds with high affinity and selectivity to the D_1^{HIGH} receptor (Ki=0.9nM) (Neumeyer et al., 1991). Recently the active enantiomer R-SKF 82957 was labeled with carbon-11 and exhibited *in vivo* binding selectivity to rat brain regions rich in D_1 receptors (DaSilva et al., 1999a). To

further characterize R-[^{11}C]SKF 82957 as a potential D_1 agonist radioligand for future use in human positron emission tomography (PET), we explored its *in vivo* binding in rats treated chronically with the full D_1 agonist SKF 81297 or with unilateral 6-OHDA lesions. Relative total densities of D_1 receptors in the rat brain were also measured with [^{11}C]SCH 23390 in these animal models exhibiting D_1 receptor supersensitivity.

4.3 MATERIALS & METHODS

Materials

R-[^{11}C]SKF 82957 and [^{11}C]SCH 23390 were synthesized as previously described (DaSilva et al., 1999a; Ravert et al., 1987). The radiochemical purity was >95% and the specific activity was >400 mCi/μmol (>14.8 GBq/μmol) at time of injection. R/S-SKF 81297 · HCl (generous gift from SmithKline Beecham Pharm., PA, USA) was dissolved in warm ethanol/propylene glycol /0.9% saline 5/10/85 (v/v/v). Desipramine hydrochloride (Research Biochemcial International (RBI), MA, USA) and pargyline hydrochloride (RBI) were dissolved in 0.9% saline. 6-OHDA · HBr (RBI) was dissolved in 0.9% saline with 0.1% ascorbic acid (Sigma, Canada) as an antioxidant. R(-)Apomorphine · HCl (Sigma) was dissolved in sterile water (Baxter Corp., Canada) containing 0.1% ascorbic acid.

Animals

The animal experiments were conducted in accordance with the recommendations of the Canadian Council on Animal Care and with approval from the Animal Care Committee at the Clarke Institute of Psychiatry. Male Sprague-Dawley rats (200-225g initial weight)

obtained from the Charles River Breeding Farm (Montreal, Canada) were utilized in all studies. Rats were housed in a 12-hour light/dark cycle with food and water *ad libitum*.

Chronic R/S-SKF 81297 Agonist Treatment

Animals were treated as described by Hu et al (1992). Twice daily injections (8h00-9h00 and 17h00-18h00) of R/S-SKF 81297 (0.5 mg/kg, s.c.) or its vehicle were given for 21 days. *In vivo* radioligand binding studies were conducted 7 days after the last agonist administration. This study was performed twice for higher statistical numbers.

Unilateral 6-hydroxydopamine Lesions

Approximately 20 min prior to surgery, the rats were injected (i.p.) with the NA re-uptake inhibitor desipramine (25mg/kg) to prevent uptake of 6-OHDA at NA terminals, and the monoamine oxidase (MAO) inhibitor pargyline (40mg/kg) to potentiate the action of 6-OHDA (Breese and Taylor, 1970). Animals were anesthetized with sodium pentobarbitol (45-50mg/kg) and placed in a Kopf stereotaxic apparatus with the incisor bar set 3.6 mm below the interaural line. The skin of the scalp was reflected and a hole was drilled in the skull over the lesion site. Unilateral lesions were made in the right medial forebrain bundle (MFB): 4.4 mm posterior, 1.2 mm lateral, 8.2 mm ventral to the surface of the skull with respect to the bregma (Paxinos and Watson, 1997). Utilizing a 30 gauge stainless steel needle, 8 µg of 6-OHDA free base (2 µg/µl solution) was injected into the MFB at a flow rate of 0.5 µl/min for 8 min. In order to prevent neurotoxin diffusion along the needle track, the needle was left in place for an additional 4 min before being withdrawn. The wound was

sutured and all animals were monitored post-operatively. Sham-lesioned control animals received 4 µl of saline with 0.1% ascorbic acid (vehicle).

Rotational Behaviour Tests in 6-OHDA Rats

Two weeks following 6-OHDA or vehicle injections, all rats were challenged with apomorphine (0.5 mg/kg, i.p.) (Gnanalingham et al., 1995c) for contralaterally directed rotations in an automated rotometer apparatus (Med Associates Inc., USA), subsequent to an initial 20 min habituation period. Rotations were measured 5 min following apomorphine administration and were terminated 60 min later. Radioligand binding studies were conducted 2 weeks after the behavioral testing.

Dopamine Detection in 6-OHDA Rats

A pilot study with 3 rats was performed to validate the stereotaxic position of the 6-OHDA lesions, the contralateral turning induced by apomorphine, and striatal DA concentrations. DA concentration was determined by HPLC with electrochemical detection using an ESA Coulochem 5100A Detector with 5011 Analytical cell and 5020 Guard cell (redox mode: DET1: +100, DET2: -390, Guard: +400 mV). Lesioned and unlesioned striatal tissues were dissected and homogenized (Biosonik, Bronwill) in 0.1 N perchloric acid, then filtered (0.45 µm nylon filter, Titan). Samples of diluted striatal extracts (100 µl) from the lesioned and unlesioned sides were sequentially injected into the analytical HPLC column (Hichrom, ODS2 5µ Spherisorb, 250 × 4.6 mm), eluted with an aqueous mixture of glacial acetic acid (0.098 M), sodium acetate (0.09 M), EDTA (0.118 mM), methanol (8%), and

sodium octane sulphonate (0.8mM), at a flow rate of 0.5 ml/min. A control run using a dopamine standard was also tested in this system for validation purposes.

In Vivo Radioligand Binding Studies

Biodistribution studies were performed as previously described (DaSilva et al., 1996b). Briefly, animals in a restraining box received 0.4-1.4 mCi in 0.3 mL of the buffered formulation of R-[^{11}C]SKF 82957 or [^{11}C]SCH 23390 by injection into a lateral tail vein (previously vasodilated in a warm water bath). All animals received approximately the same mass dose of the radioligand. Rats were sacrificed by decapitation 45 min after radiotracer administration. Blood was collected from the trunk and the brain was rapidly removed and stored on ice. For the D_1 agonist experiment, the hypothalamus, frontal cortex, olfactory tubercles, striatum, hippocampus, thalamus, rest of cortex, brain stem, and the cerebellum were excised; for the 6-OHDA experiment, the left and right striatum, olfactory tubercles, hippocampus, frontal cortex, and the whole cerebellum were dissected. All tissues were washed in saline, blotted, weighed, and counted (back corrected to the time of the first rat injection) in a gamma-counter (Cobra II, Canberra Packard) together with aliquots of the injected solution (as standards). Tails were counted in a dose-calibrator (CRC-712M, Capintec), and the injected dose corrected for residual radioactivity in the syringe and the tail. Radioactivity levels are expressed as percent of injected dose per gram of tissue multiplied by body weight (%IDK/g) to justify for differences in size. To account for possible changes to the blood-brain-barrier and blood flow brought about by the drug treatments or surgery, region-to-cerebellum ratios are also employed – the cerebellum is relatively devoid of D_1 receptors (Boyson et al., 1986) and therefore acts as a reference tissue.

Statistics

Data are expressed as mean ± SD and was subjected to a one-way ANOVA test relative to the specified controls. Differences were considered statistically significant when the probability (p) was < 0.05. N for the first group of agonist treated rats was 7 animals and 7 controls per radioligand. The second group of agonist treated rats consisted of 10 animals and 10 controls per radioligand, with only the frontal cortex, olfactory tubercles, striatum and hippocampus regions analyzed. Results from the two studies were then pooled together. For the 6-OHDA lesioning study, 13 animals and 4 sham-lesioned controls were utilized per radioligand.

4.4 RESULTS

[^{11}C]SCH 23390 and R-[^{11}C]SKF 82957 regional brain uptake following chronic agonist treatment is presented in Figure 1. Both radioligands had the highest uptake in the striatum and olfactory tubercles, two regions known to be rich in D_1 receptors (Boyson et al., 1986). As expected, the cerebellum had the lowest uptake of either radioligand. Region-to-cerebellum ratios are depicted in Figure 2. No significant changes in radioligand uptake or cerebellar ratio were detected in any brain region as compared to controls.

All 6-OHDA lesioned rats utilized in this experiment showed apomorphine- induced contralateral turns of >20 per 5 min period for a duration of 60 min. HPLC-electrochemical detection of striatal DA concentrations indicated >99% depletion in the 6-OHDA lesioned

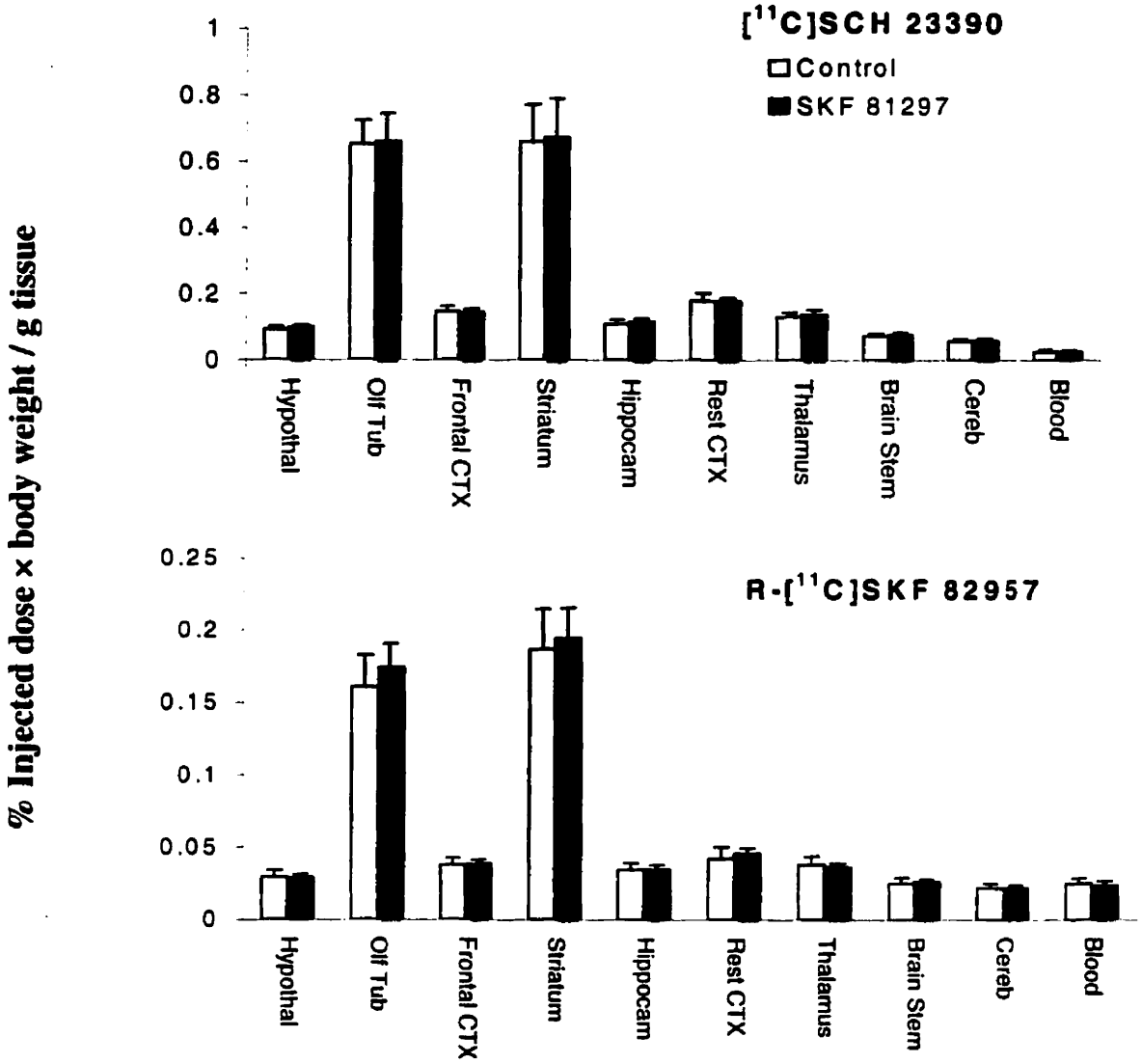

Fig. 1. Effect of chronic treatment with R/S-SKF 81297 (0.5mg/kg, s.c., twice daily injection for 21 days with 7 day withdrawal) on regional rat brain uptake of (A) [^{11}C]SCH 23390 and (B) R-[^{11}C]SKF 82957, 45 min post-injection. Data are expressed as mean of % injected dose times bodyweight in kg per gram of tissue ± S.D. N = 17 for control and treatment groups for Frontal CTX, Olf Tub, Striatum, Hippocam. N = 7 for control and treatment groups for all other brain regions. Hypothal: hypothalamus; Olf Tub: olfactory tubercles; CTX: cortex; Hippocam: hippocampus; Cereb: cerebellum.

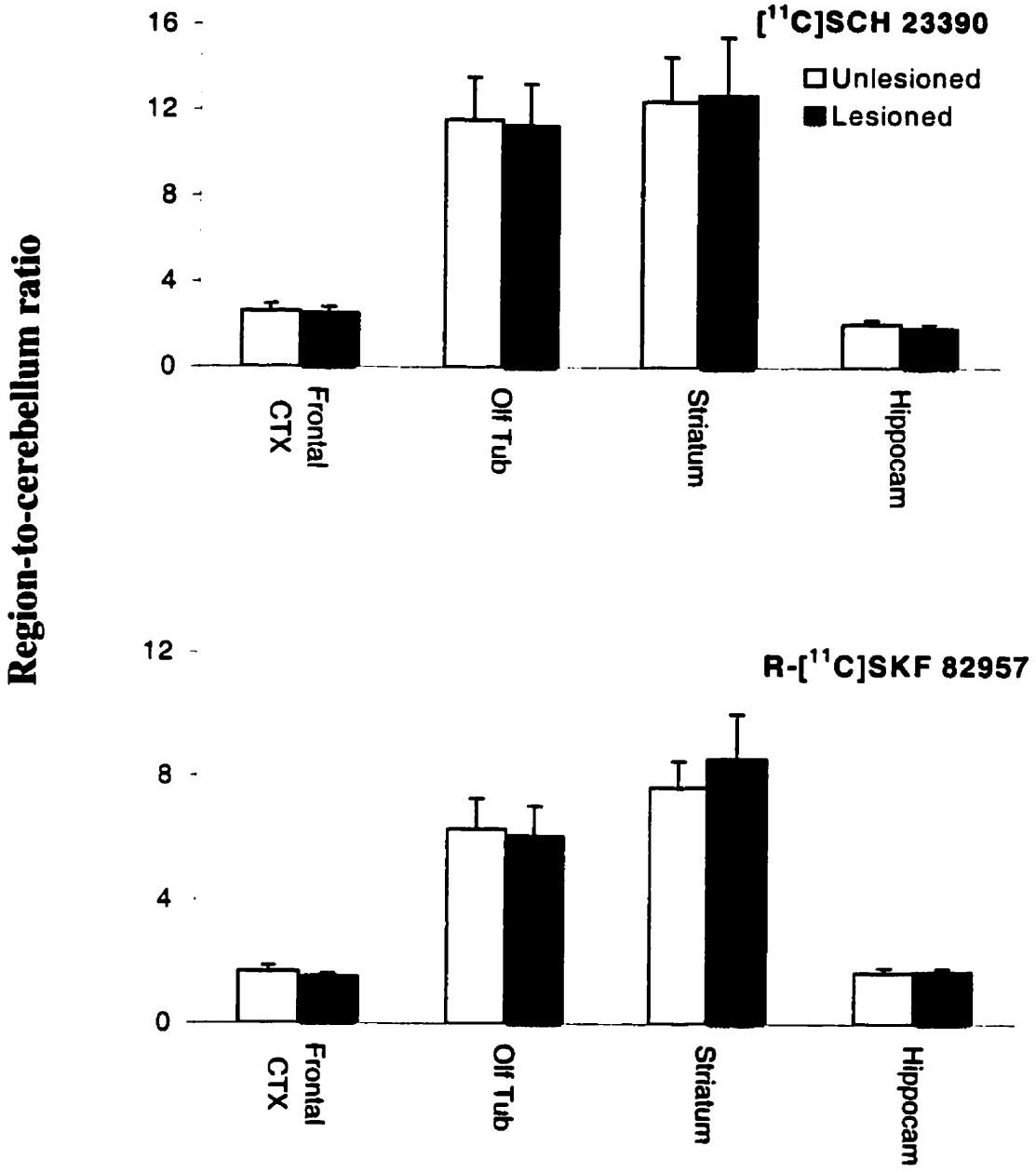

Fig. 3. Effect of unilateral sham-lesioning of the medial forebrain bundle on rat brain region-to-cerebellum ratios of (A) [^{11}C]SCH 23390 and (B) R-[^{11}C]SKF 82957, 45 min post-injection. Data are expressed as mean ± S.D. N = 4 for both radioligands. Olf Tub: olfactory tubercles; CTX: cortex; Hippocam: hippocampus.

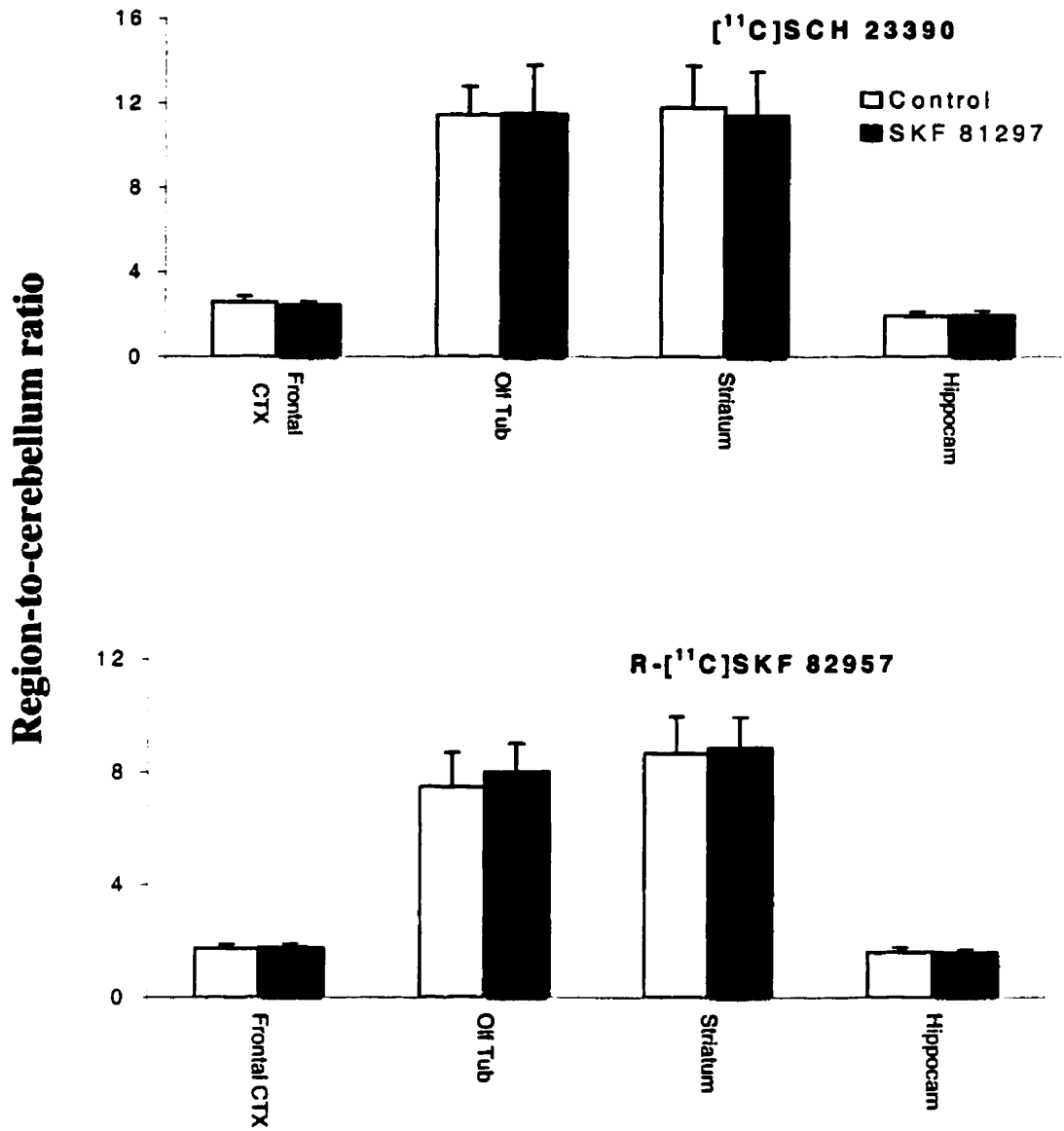

Fig. 2. Effect of chronic treatment with R/S-SKF 81297 (0.5mg/kg, s.c., twice daily injection for 21 days with 7 day withdrawal) on rat brain region-to-cerebellum ratios of (A) [^{11}C]SCH 23390 and (B) R-[^{11}C]SKF 82957, 45 min post-injection. Data are expressed as mean ± S.D. N = 17 for both controls and treatment groups. Olf Tub: olfactory tubercles; CTX: cortex; Hippocam: hippocampus

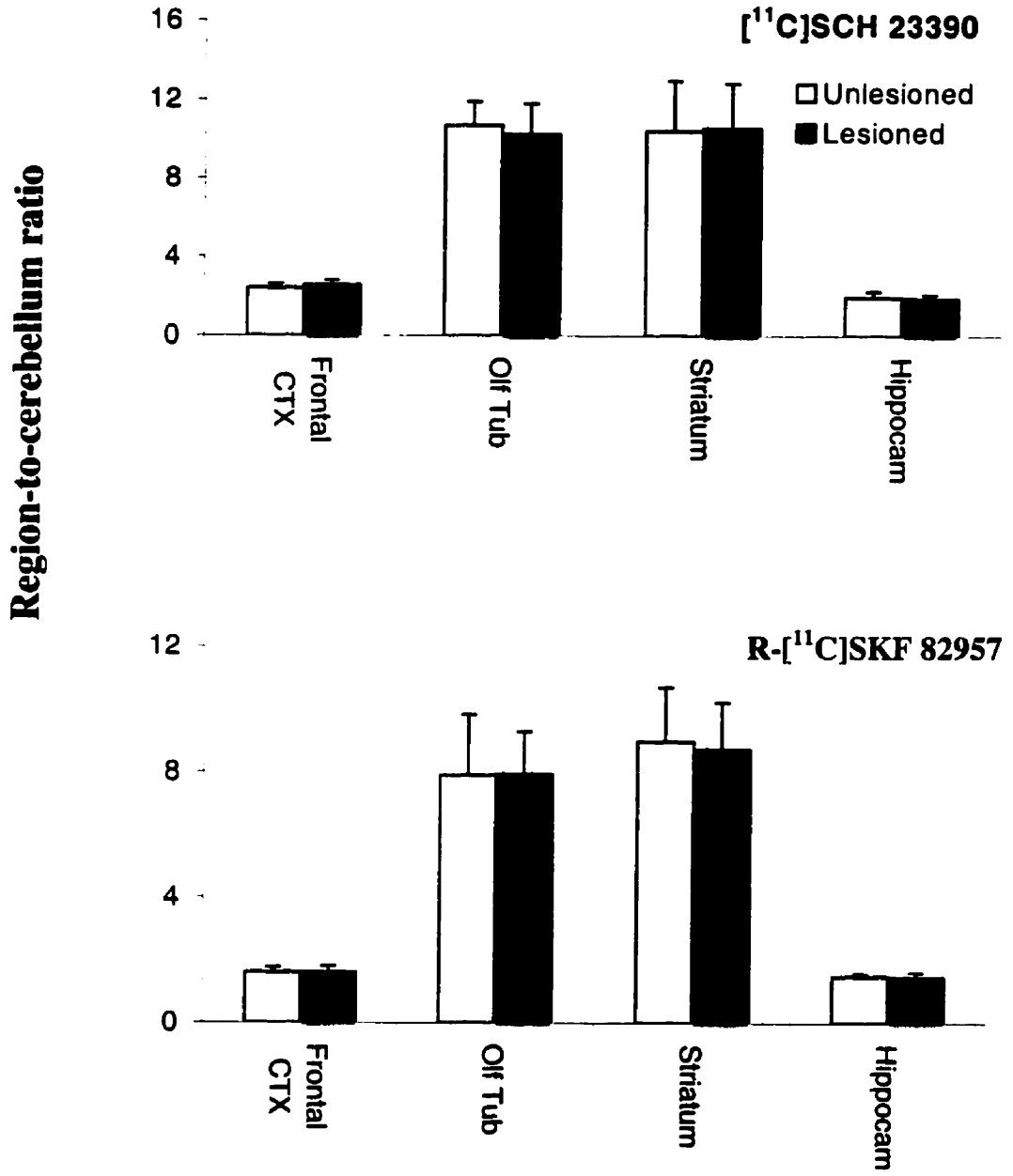

Fig. 4. Effect of unilateral 6-OHDA-lesioning of the medial forebrain bundle on rat brain region-to-cerebellum ratios of (A) [^{11}C]SCH 23390 and (B) R-[^{11}C]SKF 82957, 45 min post-injection. Data are expressed as mean ± S.D. N = 13 for both radioligands. Olf Tub: olfactory tubercles; CTX: cortex; Hippocam: hippocampus.

side (0.012±0.003 ng/ml) as compared to the non-lesioned contralateral side (1.821±0.437 ng/ml) in similarly treated rats. To ensure that any changes observed in radioligand binding are due to 6-OHDA lesioning as opposed to the surgical procedure itself, sham-lesioned animals receiving MFB vehicle injections were also studied. The region-to-cerebellum ratios for [^{11}C]SCH 23390 and R-[^{11}C]SKF 82957 in these control animals are presented in Figure 3. No significant difference is observed between the unlesioned and lesioned sides in the studied brain regions for either radioligand in these control animals. This result indicates that any changes in the 6-OHDA group would be due to the neurotoxin itself as opposed to the surgical procedure, and justifies the use of the nonlesioned side as its own control. Neither radioligand exhibited significantly altered cerebellar ratios (Figure 4) in any studied brain region between the lesioned and unlesioned control sides.

4.5 DISCUSSION

Repeated treatments with direct acting DA agonists or with indirect DA agonists, including the psychostimulant cocaine, were previously reported to produce behavioral supersensitization of the central DA system. Chronic stimulation with the D_1 agonist SKF 38393 resulted in the development of behavioral sensitization (increased stereotypy) in rats (Braun and Chase, 1988). As well, cocaine was shown to cause a significant increase in SKF 38393 induced tongue protrusions (Neisewander et al., 1996). Previous studies conducted by Hu et al (1992) showed an enhanced behavioral (e.g. grooming and oral stereotypy) and striatal inhibitory electrophysiological response to the partial D_1 agonist SKF 38393 following its chronic repeated administration. Similar results were obtained following

chronic treatment with the full D_1 agonist SKF 81297. However, this D_1 receptor mediated supersensitivity was only observed after a one-week withdrawal period, and was abolished one month after treatment termination. In these rats, a subsensitivity to SKF 38393 was observed at 6-10 hours after treatment, with the electrophysiological results closely matching the behavioral results (Hu et al., 1992; White et al., 1990). In our study, no significant difference was observed in the R-[^{11}C]SKF 82957 or [^{11}C]SCH 23390 uptake or region-to-cerebellum ratios between the treated (chronic SKF 81297) and the control groups in any brain region. Preceding research by this lab group using the same radioligands obtained identical results in rats after subchronic and chronic cocaine treatment paradigms with various lengths of withdrawal periods (Greenwald et al., 1999b). These results suggest that the D_1 supersensitivity obtained following chronic direct or indirect D_1 agonist stimulation is not due to receptor changes as observed *in vivo* with [^{11}C]SCH 23390 and R-[^{11}C]SKF 82957.

Other *in vitro* binding studies also indicate no change in the total density of D_1 receptors following similar DA agonist drug treatment stratagems (Braun and Chase, 1988; Lappalainen et al., 1992; Matsuda et al., 1992; Neisewander et al., 1991). A possible explanation for these findings is that the supersensitization associated with these treatments is caused by changes downstream of the D_1 receptor in the signal transduction pathway. For example, the levels of Gi are reportedly reduced following chronic cocaine treatment (Nestler et al., 1990; Striplin and Kalivas, 1993), rendering the D_1-Gs complex more efficacious at stimulating AC, and thus producing an enhanced response to D_1 agonist stimulation. It is important to note here that [^{11}C]SCH 23390 and R-[^{11}C]SKF 82957 displayed different *in vivo* striatal binding following chronic SCH 23390 (Greenwald et al., 1999a) and reserpine

treatments in rats (DaSilva et al., 1998a), suggesting that the two radioligands bind to a distinct subpopulation of D_1 receptors that are differentially regulated.

The unilateral 6-OHDA animal model of hemiparkinsonism was employed in this study because of its ability to cause denervation supersensitivity of the D_1 receptor. 6-OHDA lesions of the MFB results in the loss of nigrostriatal fibers as observed by a decrease in dopamine transporter sites at the striatum (Przedborski et al., 1995). This type of denervation is well-characterised and can be imaged in living human hemiparkinsonian brains using the DA transporter radiotracer [^{11}C]RTI-32 and PET (Guttman et al., 1997). In unilateral 6-OHDA lesioned rats the striatal dopaminergic cell loss is well correlated to apomorphine induced contralateral turning (Gnanalingham et al., 1995c; Hossain and Weiner, 1995; Ungerstedt, 1971a). All animals used in this study displayed a rate of contralateral turning that is correlated to >95% loss of DA uptake sites (Gnanalingham et al., 1995c). Furthermore, in this study, HPLC analysis of striatal DA concentrations demonstrated a >99% loss of ipsilateral striatal DA as compared to the unlesioned side. The fact that these animals show contralateral turning after direct agonist receptor stimulation indicates that the DA receptor system in the striatum is supersensitive on the lesioned side (Ungerstedt, 1971c).

In the present study, no change in the uptake or the region-to-cerebellum ratios of [^{11}C]SCH 23390 in any brain region was detected, indicating no alteration in the relative total density of D_1 receptors between the lesioned and unlesioned sides. Discrepancies regarding the total D_1 receptor density following unilateral 6-OHDA lesioning exist in the literature with no change (Graham et al., 1990; Lawler et al., 1995; Trugman et al., 1990), and increases or decreases reported (Iwata et al., 1996; Joyce, 1991; Porceddu et al., 1987). In

the present study no change in R-[^{11}C]SKF 82957 binding was also observed between the lesioned and nonlesioned sides. These results suggest that the behavioral supersensitization associated with the D_1 receptor is due to modifications at sites downstream to the receptor. Indeed, there is evidence to support this hypothesis. An increase in agonist induced activity of AC has been noted in several studies (Gnanalingham et al., 1995c; Pifl et al., 1992b; Pinna et al., 1997). Theoretically, this elevated AC activity can account for the enhanced responsiveness. Other processes have also been implicated, such as an increase in the Gs protein in the rat basal ganglia following unilateral 6-OHDA lesioning (Marcotte et al., 1994; Tenn and Niles, 1997).

Hervé et al (1992) utilizing autoradiographic localization of [^{3}H]DA and [^{3}H]SCH 23390, reported a 60-81% decrease in D_1^{HIGH} receptor sites in the 6-OHDA lesioned striatum, with no change in the D_1 Bmax. This experiment was performed 6 weeks post lesioning. The same group also identified an increased Gs protein expression following identical neurotoxin lesioning (Hervé et al., 1993). However, mass action law applied to the ternary complex theory of receptor kinetics (De Lean et al., 1980; Mackay, 1990) suggests that elevated G protein levels would be associated with an increase in the proportion of receptors in the high-affinity state, contrary to what was observed experimentally in the Hervé et al (1992) study. In fact, experiments confirming this increase in the proportion of high-affinity receptors was demonstrated in reconstituted phopholipid vesicles, showing that the proportion of D_2^{HIGH} receptors was increased with increasing G protein concentrations (Ohara et al., 1988). Contrary to the above results, Pifl et al (1992) noted that 6-OHDA denervation resulted in a stabilized NaCl-insensitive form of the D_1^{HIGH} receptor. However the mechanism of this D_1^{HIGH} insensitivity to NaCl, its stabilization, and the exact

physiological relevance remains uncertain. More recently Cai et al (1998) demonstrated an enhanced D_1 receptor/Gs_α protein coupling, as determined by examining [^{3}H]SCH 23390 and Gs_α antibody binding in immunoprecipitates of striatal membranes prepared from the 6-OHDA lesioned hemisphere. This enhanced coupling is analogous to the D_1^{HIGH} receptor, suggesting an increase in the proportion of D_1^{HIGH} in the absence of an increased D_1 Bmax. The inconsistencies between our results and the reported results may be partially explained by the radically different method of receptor labeling (*in vitro* versus *in vivo*) and the radioligands used.

In summary, the development of behavioral supersensitization is a complex phenomenon involving possible changes throughout the different components of the receptor-signal transduction pathway. Although no significant changes in the uptake and cerebellar ratios for either [^{11}C]SCH 23390 or R-[^{11}C]SKF 82957 were found in rats following treatment with the direct acting D_1 agonist SKF 81297 or with unilateral 6-OHDA-lesioning, it does not preclude the existence of non-receptor mediated modifications. As discussed, such downstream changes in the signal transduction pathway may account for the enhanced responsiveness to agonist stimulation.

4.6 STATEMENT OF SIGNIFICANCE

This paper tested our working hypothesis that the binding of R-[^{11}C]SKF 82957 is increased in the rat striatum following pharmacological manipulation known to cause D_1 receptor supersensitization, while that of [^{11}C]SCH 23390 is unchanged. The results demonstrate no change in the regional brain uptake and cerebellar ratios of either radioligand under either treatment paradigm. This suggests that other events, downstream of the D_1

receptor, are responsible for the supersensitized response to D_1 stimulation observed in previous studies

5.0 SUMMARY AND GENERAL DISCUSSION

5.0 SUMMARY AND GENERAL DISCUSSION

The ultimate objective of this research project is to use R-[^{11}C]SKF 82957 as an *in vivo* marker of the D_1 high-affinity state in humans with PET. Until recently all research on the high- and low-affinity state of DA receptors in both animal models and human neuropsychiatric diseases has been performed *in vitro*. Unfortunately these techniques are not suitable for measuring receptor kinetics throughout disease progression or during pharmacological/surgical intervention. Conversely, the use of a non-invasive tool, such as PET, is appropriate for such assessment.

PET imaging of the D_1^{HIGH} receptor requires the use of a selective agonist. The reason being that antagonists cannot determine differences in the high- versus the low-affinity state. Therefore, D_1 antagonist radioligands are used in PET to study the total receptor density. In the past, the antagonist [^{11}C]SCH 23390 has been successfully employed to image the D_1 receptor in a number of human neuropsychiatric diseases with PET (Kent et al., 1980; Rinne et al., 1990b; Suhara et al., 1991).

To date no selective D_1 agonist radioligand has been developed for PET. R-SKF 82957 was chosen as a possible candidate for several reasons. First, it binds *in vitro* with high affinity and selectivity to the high-affinity state of the D_1 receptor (Neumeyer et al., 1991); second, it is an agonist of the receptor such that stimulation of AC occurs (Pfeiffer et al., 1982); third, the availability of the precursor molecule R-SKF 81297; and fourth, the possibility to label this molecule with [^{11}C]CH_3I. R-[^{11}C]SKF 82957 is now routinely synthesized in our lab with high yields, specific activity and purity. In addition, *in vivo* studies by this laboratory have previously shown in rats that R/S-[^{11}C]SKF 82957 has rapid

brain uptake and is selective for D_1 receptors (DaSilva et al., 1996a; 1996b). But before such a compound can be administered to humans for PET research, it is necessary to establish its pharmacokinetic profile in rats and assess its ability to measure *in vivo* changes to the D_1 receptor in treatment paradigms known to cause receptor supersensitivity. This analysis was one of the purposes of the research presented in this thesis.

Neuroreceptor imaging by PET requires that the radiotracer have the ability to cross the BBB and accumulate within the intended target organ with little non-specific binding. The most desirable PET radiotracer would be one with a slow formation of metabolites that do not penetrate the BBB (Foged et al., 1996). It is necessary to identify the radiotracer and its potential radioactive metabolites in the plasma, as well as to determine if any cross the BBB and have affinity for neuroreceptors. Pharmacokinetic modelling utilizing an arterial input function would be required if such a radioactive metabolite were detected. Arterial input functions, obtained from the measured radioactivity in plasma (Swahn et al., 1992), would be used to produce mathematical corrections that take into account the fraction of radiolabeled metabolites in plasma.

Chapter 2 examined the pharmacokinetic profile of R-[^{11}C]SKF 82957 in rats. Plasma studies indicated a low presence of metabolites with ~86% of the injected compound unchanged 30 min post-injection. Previous studies with the similar benzazepine analog, SCH 23390, revealed that the major liver metabolites present in plasma were the conjugated O-sulphate and O-glucuronide derivatives (Swahn et al., 1994; Tephly et al., 1994). These hydrophilic metabolites are not likely to cross the BBB. Similar liver metabolites are expected to be produced with R-[^{11}C]SKF 82957 but in a lower extent due to the low presence of radiolabeled metabolites in plasma. It would also be anticipated that these O-

sulphate and O-glucuronide conjugates of R-[^{11}C]SKF 82957 would not cross the BBB. In order to verify this, rat brain homogenates were examined for the presence of radiolabeled metabolites. At 30 min post-injection, only a single peak corresponding to the pure radioligand was observed by TLC analysis, even though polar metabolites had been detected in the blood. These results support our working hypothesis that the radioactive signal observed in the rat brain is due to unchanged R-[^{11}C]SKF 82957 and not to radioactive metabolites. The C_{18} Sep Pak extraction method was optimised such that the water phase would consist of all hydrophilic polar metabolites and the organic elutant would comprise the unchanged compound and any non-polar metabolites. Only the organic elutant was analysed by TLC, because only this fraction was expected to contain non-polar radiolabeled metabolites with the potential to cross the BBB. The TLC assay was optimised such that separation of the normethyl-SKF 82957 from that of SKF 82957 could be achieved.

Chapter 3 outlines the results obtained on human volunteers following intravenous R-[^{11}C]SKF 82957 injection and PET image acquisition. The use of carbon-11 ($t_{1/2}$ = 20.4 min) labeled radiotracers limits PET experiments to approximately 90 min. Because of this time constraint only metabolism that occurred within this interval was examined. A low accumulation of metabolites in human plasma was observed with > 85% unchanged R-[^{11}C]SKF 82957 at 80 min post injection. As in rat plasma, only a single radioactive peak on TLC corresponding to unchanged R-[^{11}C]SKF 82957 was detected in the organic fraction from the Sep Pak elution of human plasma samples, taken at various time points throughout the PET scan. In principle, the lower presence of metabolites in humans is a benefit as it decreases the possibility of them crossing the BBB and interfering with R-[^{11}C]SKF 82957 binding in the brain. The high specific activity of the radiotracer (>1300 Ci/mmol,

corresponding to <4.5 μg of free base product) allows for the administration of a very small amount of unlabeled compound. This further renders the presence of unlabeled metabolite interference with R-[^{11}C]SKF 82957 binding likely unproblematic. In fact, unlabeled metabolites would not saturate the D_1 receptors, since they could only occupy a very small percentage of these sites.

High radioactivity was detected in the D_1 rich striatum and, to a lesser extent the cortex, following R-[^{11}C]SKF 82957 injection in humans, which is in agreement with previous results obtained in rats (DaSilva et al., 1999b; 1996b) and with the working hypothesis of this thesis. As with other D_1 PET radioligands, the BP of R-[^{11}C]SKF 82957 decreased with age (~9% per decade), indicating a probable reduction in the SKF 82957 binding site over time. Note that discrepancies in the literature exist as to whether a decrease in the D_1^{HIGH} receptor occurs. Previous *in vitro* competition studies using [^{3}H]SCH 23390 and postmortem human brains have shown a decrease in D_1^{HIGH} with age in the frontal cortex (De Keyser et al., 1990), as well as no change with age in the putamen (De Keyser et al., 1990). Our results, however, are the first reported from *in vivo* human subjects using a DA agonist radioligand, and concur with our working hypothesis.

Scan-rescan data was used to assess both the reproducibility and reliability of our PET data acquisition technique. ANOVA tests indicated no change in the BP between scan one and scan two, thus confirming the reproducibility of our results. In order to determine how reliably these results can be obtained with our measurement techniques, inter- and intra-rater reliability correlation coefficients were assessed. This indicated that ROI determination and R-[^{11}C]SKF 82957 BP calculation could be acquired with high inter- and intra-rater reliability. This again fulfils the scope of our working hypothesis.

The only limitation to the use of R-[^{11}C]SKF 82957 for PET scans remains its relatively low signal-to-noise ratio. For example, a 45 year old healthy human subject scanned with [^{11}C]SCH 23390 is expected to have a BP (Lammertsma) of approximately 1.5 (unpublished data). In contrast, the same subject scanned with R-[^{11}C]SKF 82957 would have a much lower BP of 0.48. These differences may be exaggerated when subjects of differing brain disorders are examined due to altered brain uptake and increased variability in receptor binding such that lower signal-to-noise ratios can be created. Previous *in vitro* studies examining the proportion of D_1^{HIGH} receptors indicate that they represent 20-40% of the total receptor population (De Keyser et al., 1988; Mamelak et al., 1993; Rubinstein et al., 1990; Sidhu et al., 1991). Hypothetically, our data on the same 45 year old subject comparing R-[^{11}C]SKF 82957 binding to that of [^{11}C]SCH 23390 produces a D_1^{HIGH} proportion of 30%. As noted, this value agrees with published reports, although other factors, such as dissociation affinity, must also be addressed.

In order to further evaluate the *in vivo* binding characteristics of R-[^{11}C]SKF 82957, we chose to use two rat models known to cause D_1 receptor supersensitivity. Two hypotheses in this thesis state that R-[^{11}C]SKF 82957 (1) binds *in vivo* to the high-affinity state of the D_1 receptor, and (2) that its receptor binding may be differential regulated as compared to the D_1 antagonist [^{11}C]SCH 23390, in response to dopaminergic manipulation. Receptor supersensitivity may arise by several different mechanisms. We chose to explore that of enhanced coupling between the D_1 receptor and the G protein such that an increase in the D_1^{HIGH} receptor occurs. Chapter 4 detailed the experiments performed and the results obtained.

Experiments using chronic D_1 agonist treatment have shown receptor supersensitization following a specified withdrawal period (Braun and Chase, 1988; Neisewander et al., 1996). With our treatment stratagems, Hu et al (1992) has shown an enhanced inhibitory effect of SKF 81297 following its 21 day chronic administration, and a one-week withdrawal period, on striatal electrophysiological responses. Our results indicated no change in either the regional brain uptake or the region-to-cerebellum ratios of [^{11}C]SCH 23390 or R-[^{11}C]SKF 82957 as compared to controls. This is in contrast to our working hypothesis which predicted an increase in the binding of R-[^{11}C]SKF 82957 with or without a change in the receptor Bmax as measured by [^{11}C]SCH 23390. As explained in chapter 4, the resultant behavioural supersensitivity probably occurred due to changes in the signal transduction pathway below that of the receptor. These changes would therefore not be quantified by our methodology, but could explain the electrophysiology results obtained by Hu and colleagues (1992). It is possible that by increasing the dosage regime such a receptor modification as predicted by our working hypothesis would be observed. However, to do so would risk the introduction of D_1 associated proconvulsant activity in rats (Al-Tajir et al., 1990; Hubbard and Trugman, 1993; Starr and Starr, 1993). This is an unacceptable risk because it not only injures the animals but acts as well as a confounding variable, since control rats treated with vehicle could not be induced into seizure. It must also be noted that improper formulation of SKF 81297 is unlikely to have contributed to these results since the solution was freshly prepared daily, and all rats that received this drug displayed the appropriate grooming and oral stereotypy characteristic of this D_1 agonist treatment (Hu et al., 1992; Molloy and Waddington, 1984; White et al., 1990).

Previous studies with the unilateral 6-OHDA-lesioning model have consistently demonstrated D_1 receptor supersensitivity. This is seen behaviourally as increased contralateral turning upon D_1 receptor stimulation, and biochemically as an increase in AC activity. The benefit of utilizing the unilateral 6-OHDA rat animal model is that the unlesioned side of the same animal acts as a within subject control. That is to say that all comparisons are made between the lesioned and unlesioned side within the same rat. To verify that our lesioning technique does not change the radioligand uptake or region-to-cerebellum ratios, unilateral sham-lesioned controls were also tested. As stated in chapter 4, R-[^{11}C]SKF 82957 and [^{11}C]SCH 23390 binding was unchanged between the lesioned and unlesioned hemispheres in these controls, therefore insuring that any changes observed in the 6-OHDA lesioned animals is due to dopaminergic denervation and not the surgical procedure. To guarantee sufficient dopaminergic degeneration, a pilot study consisting of 12 animals was first conducted. This was needed in order to verify that proper lesioning coordinates had been obtained. The MFB was selected as the target site because all nigrostriatal fibres are tightly packed together there, and therefore increase the likelihood of their destruction following 6-OHDA injection (Mokry, 1995; Paxinos and Watson, 1997). Three possible coordinates were chosen, and surgeries were performed with these sites in 4 rats each. Two weeks following surgery these animals were tested in an automated rotometer for contralateral turning following apomorphine (0.5 mg/kg, i.p.) challenge.

Apomorphine was chosen to determine the extent of rotation instead of amphetamine because of its ability to better predict striatal degeneration (Hefti et al., 1980; Hossain and Weiner, 1995; Hudson et al., 1993). Furthermore, apomorphine acts directly at the DA receptors, therefore providing strong evidence that the post-synaptic receptors on the lesioned

striatum are supersensitive. Contrary to this, amphetamine acts pre-synaptically to increase DA release and enhance the receptor response at the innervated striatum, not the lesioned side. Final lesioning coordinate selection was based upon the extent of contralateral rotation, which previously had been shown to correlate to >95% loss of ipsilateral striatal DA uptake sites (Gnanalingham et al., 1995c), and the results of HPLC-electrochemical detection which indicated >99% DA loss in the lesioned striatum. The brain regions of interest were chosen based upon the following criteria: (1) the striatum, because it is the site of supersensitivity, and because it acts as an animal model of the denervation seen in PD; (2) the olfactory tubercles, since they contain D_1 receptors that should not be affected by MFB lesioning and therefore would act as negative controls; (3) the frontal cortex is also of interest because it contains D_1-like receptors and is therefore routinely assayed, however no predictions were assumed for this region; (4) the hippocampus; and (5) cerebellum, which function as negative controls. The cerebellum is also used as the region of nonspecific binding when calculating the cerebellar ratios.

Our working hypothesis states that with the loss of striatal DA, and in the absence of receptor upregulation, the D_1 mediated striatal supersensitivity may be due to an increase in the proportion of receptors in the high-affinity state. As discussed in chapter 4, binding studies with R-[^{11}C]SKF 82957 and [^{11}C]SCH 23390 in 6-OHDA-lesioned rats indicated no significant change between the nonlesioned and lesioned side. Other laboratories using *in vitro* techniques have corroborated these results (Pifl et al., 1992b), yet discrepancies in the literature exist (Cai et al., 1998; Hervé et al., 1992). Our results suggest that the cause of 6-OHDA supersensitivity lies downstream of the D_1 receptor, the mechanism of which could involve amplification of other components of the signal transduction system.

One possible explanation for the apparent disagreement between our results and those of other research groups could lie in the method of investigation. Our *in vivo* assay approach differs radically from *in vitro* studies. The use of physiologic buffers and incubation media might not replicate the complexity of the D_1 receptor-G protein binding interaction with an agonist. Recent investigations have added to the discrepancies between *in vitro* and *in vivo* techniques. For example, Roseboom and Gnegy (1989) utilized standard *in vitro* binding assays with [^{3}H]SCH 23390, [^{3}H]DA, and Gpp(NH)p (nonhydrolyzable GTP analog) to study the D_1^{HIGH} receptor following acute amphetamine challenge. The results indicated no change in the D_1 receptor Bmax, but a 30% decrease in the proportion of D_1^{HIGH} receptors in the striatum, 30 min post-injection. These findings concur with the current ternary model of receptor kinetics, however they may be contrasted with those of our lab. Studies on rats performed using the same methods as reported in chapter 4 revealed no change in the R-[^{11}C]SKF 82957 striatum-to-cerebellum ratio following acute amphetamine challenge (Greenwald et al., 1999a). [^{11}C]SCH 23390 binding was also unchanged. Similarly, Laruelle et al (1998) showed no change in the striatal binding of R-[^{11}C]SKF 82957 and [^{11}H]SCH 23390 in baboons that were subjected to an acute amphetamine challenge and examined *in vivo* by PET. Note that in each of these cases it is the putative high-affinity state results that are consistently different between the *in vitro* and *in vivo* studies, while the results of total density binding showed no change between all studies published to date. This indicates that both techniques can be reliably used to measure total receptor changes. However the dynamic nature of the high-affinity receptor state requires careful experimentation to ensure that physiological research conditions exist. Clearly more work is

needed to explore the cause of these discrepancies when attempting to assay the D_1^{HIGH} receptor by *in vivo* versus *in vitro* techniques.

The findings of this thesis may be summarised as follows:

1. R-[^{11}C]SKF 82957 has a low presence of radiolabeled metabolites in rat and human plasma.
2. No radiolabeled metabolite crosses the rat BBB.
3. R-[^{11}C]SKF 82957 binds in humans to the D_1 rich striatum and displays a decreased BP with age.
4. R-[^{11}C]SKF 82957 can provide reproducible and reliable PET data in human brains.
5. Binding studies performed following chronic agonist treatment or 6-OHDA lesioning in rats failed to indicate a significant difference in the uptake or region-to-cerebellum ratios of either [^{11}C]SCH 23390 or R-[^{11}C]SKF 82957 as compared to controls.

These findings are the first step required to assess the value of R-[^{11}C]SKF 82957 as a potential PET agonist radioligand. PET studies conducted to date have relied on the use of antagonists that cannot measure this receptor state. However the use of *in vitro* approaches have hinted at the possibility that changes in the D_1 high-affinity state do occur with disease. For example, Mamelak et al (1993) showed a significant increase in the proportion of D_1^{LOW} receptors and a significant enhancement in the affinity of D_1^{HIGH} in postmortem studies on human SZ sufferers. Likewise, Rubinstein et al. (1990) presented a 51% increase in the proportion of D_1^{HIGH} following chronic reserpine treatment in mice. To date, R-[^{11}C]SKF 82957 shows excellent promise in assessing these types of changes at the D_1 receptor. As

such it would provide a unique and invaluable tool for the study of human neuropsychiatric diseases, including SZ, PD, TD, HD and drug addiction, using PET.

6.0 CONCLUSIONS

6.0 CONCLUSIONS

R-[^{11}C]SKF 82957 is the first selective PET radioligand of the DA system capable of imaging the G protein coupled DA receptor complex. The metabolic profile of this compound in both rats and humans indicates a low presence of metabolites in plasma, and that no radiolabeled metabolites should interfere with PET brain imaging. As well, the scan data from the human volunteers provided reproducible and reliable results. In principle, this makes R-[^{11}C]SKF 82957 appropriate for PET imaging of D_1 receptors. However, more work is required before this radioligand can be utilized in broader research studies. In future human PET studies, it is important that the scan reproducibility of this tracer be assessed in neuropsychiatric subjects, not only in controls. The need to assess these subjects arises from the fact this population is expected to have greater variability in radioligand binding, and possibly an altered brain uptake as compared to controls. For example, it would be anticipated that PD patients would have increased R-[^{11}C]SKF 82957 binding as compared to controls due to the decrease in dopaminergic innervation. In contrast, SZ subjects would be expected to have a lower R-[^{11}C]SKF 82957 binding as compared to controls, due to the overstimulation of the DA receptor system.

In order to further evaluate the ability of R-[^{11}C]SKF 82957 to measure *in vivo* changes at the probable D_1^{HIGH} receptor, we selected two animal models known to cause D_1 receptor supersensitization. Unfortunately, no statistically significant change in R-[^{11}C]SKF 82957 binding was observed with either the chronic SKF 81297 agonist treatment or the 6-OHDA-lesioning model as compared to controls. However, previous studies by this lab have shown changes in R-[^{11}C]SKF 82957 binding following specific pharmacological treatment.

Chronic reserpine treatment in rats caused a reduction in the striatum-to-cerebellum ratio of R-[^{11}C]SKF 82957 which was significantly less pronounced then that of [^{11}C]SCH 23390, suggesting that the chronic reserpine treatment increased the proportion of receptors in the high-affinity state, with a concomitant decrease in receptor density. A possible explanation for these results are that a downregulation in D_1 total density occurred due to a decrease in the animals body temperature (Sidhu and Kimura, 1994), and this effect may have been less prominent at D_1^{HIGH}. The abstract of this study is included in the appendix of this thesis. As well, chronic SCH 23390 therapy in rats demonstrated an increase in striatal- and olfactory tubercle-to-cerebellum ratios with [^{11}C]SCH 23390, while no change was observed with R-[^{11}C]SKF 82957. Hess et al (1986a) found similar results utilizing an *in vitro* competition study, with no change in the proportion of striatal D_1 receptors in the high-affinity state following chronic SCH 23390 treatment in rats. These experiments demonstrate that R-[^{11}C]SKF 82957 is capable of measuring a D_1 receptor subpopulation distinct from that of [^{11}C]SCH 23390. Future studies will assess D_1 receptor changes with R-[^{11}C]SKF 82957 and [^{11}C]SCH 23390 following chronic D_2 receptor antagonism and ethanol treatments in rats.

The ultimate objective of this research group is to develop an agonist PET radioligand that can selectively measure the high-affinity state of the D_1 receptor. R-[^{11}C]SKF 82957 shows great promise in this field, however it is not the only contender. Other benzazepine agonist analogues exist which show similar pharmacodynamic properties and could prove more efficacious for this application. However, to date, R-[^{11}C]SKF 82957 is the only dopamine PET agonist radioligand of its kind which shows accumulation of radioactivity in the human striatum. The next most important step in its development will be to use this tracer in human subjects suffering from neuropsychiatric disorders. This research project

opens a new window in PET imaging, that of possibly visualizing the functional D_1 high-affinity sites *in vivo* in humans

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8.0 APPENDIX

8.1 Chronic Reserpine Differentially Alters *In Vivo* Binding of D_1 Agonist R/S- and R-[^{11}C]SKF 82957 as Compared to [^{11}C]SCH 23390 in Rat Brain

J.N. DaSilva, E. Greenwald, R.A. Schwartz, A.A. Wilson and S. Houle

PET Centre, Clarke Institute of Psychiatry, Depts. of Psychiatry and Pharmacology, Univ. of Toronto, Toronto, Ontario, Canada, M5T 1R8.

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R/S- & R-SKF 82957 are D_1 agonists that bind with high affinity and selectivity to the high-affinity state of D_1 receptors. *In vivo* evaluation of R/S- and R-SKF 82957 labeled with C-11 demonstrated high binding selectivity for D_1 receptors in rat brain regions rich in D_1 receptors, such as the striatum (Str) (Life Sci. 58:1661-1670, 1996; J. Nucl. Med. 38:76p, 1997). Previous studies have shown that chronic administration of reserpine for five days depletes dopamine stores by ~98%, rendering D_1 receptors supersensitive and increasing the proportion of D_1 receptors in the high-affinity state, as measured *in vitro*. Enhancement of the coupling efficiency of stimulatory G-protein without changing D_1 receptor density is also reported in this animal model of Parkinson's disease. Reserpine-treated rats (5 daily injections of 1 mg/kg, s.c.; controls received vehicle only, s.c.) were injected with R/S-[^{11}C]SKF 82957 (n=7), R-[^{11}C]SKF 82957 (n=8) and [^{11}C]SCH 23390 (n=14), 2 h after the last reserpine administration. Animals were sacrificed at 45 min post-injection of the tracers. Brain uptake (expressed as % of injected dose x body weight per g of tissue) of R/S- & R-[^{11}C]SKF 82957 was more affected by reserpine as compared to [^{11}C]SCH 23390. Compared to controls, Str-to-cerebellum (Cer, devoid of D1 receptors) ratios were significantly reduced in reserpine-treated rats by 36% with [^{11}C]SCH 23390, and only 22% and 26% with R/S- & R-[^{11}C]SKF 82957, respectively. The Str-to-Cer ratios of R/S- & R-[^{11}C]SKF 82957 were significantly ($p<0.07$) less reduced than that of [^{11}C]SCH 23390, suggesting that the repeated-reserpine treatment proportionately increased the binding sites of [^{11}C]SKF 82957 as compared to [^{11}C]SCH 23390. These results suggest that chronic reserpine increases the proportion of D_1 receptors in their high-affinity state in the striatum as measured *in vivo* with R/S- & R-[^{11}C]SKF 82957.